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EFFECT OF BURNING ON FOREST SOILS[1]

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Estimates place the area burned over annually by forest fires at seven million acres (4). Damage to the timber is seldom questioned. Damage to the soil through destruction of humus-making litter on the forest floor likewise appears to be a problem of importance. How much good humus material is destroyed and how much valuable plant food is lost annually by fires cannot be estimated.

Studies by other workers have indicated that the litter and partially humified material on the forest floor may contain 2 per cent or more of nitrogen (2). All the nitrogen is lost when organic matter burns. Under some conditions this loss may amount to several hundred pounds an acre, as indicated by work done in Minnesota (2).

There are, however, certain compensating effects which follow burning. Ash is liberated and becomes an immediate source of available nutrients. Burning destroys organic acids and liberates bases which further neutralize acids in the soil, thus improving conditions for biological processes and plant growth.

Decomposition of leaf litter or any high carbon material results usually in slow production of available nitrate. Fungi which are especially active in the "F" layer produce acids and thus discourage nitrate-forming organisms. Nitrifying organisms are most active in neutral or alkaline soils. These same fungi need nitrogen for their own cell protoplasm during growth. Any nitrate that is produced, therefore, is likely to be consumed by organisms.

Burning changes the soil flora and makes conditions favorable for bacteria rather than fungi. A different type of process therefore occurs. Probably more of certain nutrients become available for promotion of biological action and plant growth. The present paper deals with some of these questions.

METHOD OF STUDY

Four soils representing samples taken to a depth of 6 inches were studied. The soil, which was obtained from Alsea Mountain of the Coast Range in Western Oregon, has been classed as Olympic clay. Soil A was taken from a stand of young Douglas fir (*Pseudotsuga taxifolia* Lamb Britt) reproduction

[1] Published as technical paper No. 203 with the approval of the director as a contribution from the soils department, Oregon Agricultural Experiment Station.

[2] Graduate student in forestry and associate professor of soils, respectively.

about 10 years old. The ground cover was principally bracken fern (*Pteridium aquilinum pubescens* Underw.), with some leaf litter from such brush as vine maple (*Acer circinatum* Pursh) and hazel (*Corylus rostrata* Ait. Var. California A. DE.). Soil B was taken from an area heavily burned in the summer of 1932, when all litter was destroyed. Soils A and B were adjacent, being separated by the highway which stopped the fire. Soil C came from a stand of old growth Douglas fir with a dense ground cover of salal (*Gaultheria shallon* Pursh). Soil D was from a 1930 "burn" now covered with fire weed (*Epilobium angustifolium* L.), bracken fern, and salal.

Samples of each of the soils were weighed into tumblers in duplicate and treated as follows:

1. Nothing (check).
2. Sulfate of ammonia at the rate of 300 parts of nitrogen per million of soil.
3. Sulfate of ammonia at the same rate and precipitated calcium carbonate[3] at the rate of 2,000 pounds per million pounds of soil.
4. Ground Douglas fir litter at the rate of 5,000 pounds per million of soil.
5. Litter and sulfate of ammonia at the above rates.
6. Litter, sulfate of ammonia, and precipitated calcium carbonate at the same rates as above.

Twenty-five per cent of moisture was added to the air-dry soils, and the tumblers were incubated at room temperature for a period of 12 weeks. Moisture was maintained by frequent additions of water. At each interval of 4 weeks soil samples were drawn and nitrates determined by the phenoldisulfonic acid method. At the end of the period water-soluble calcium and potassium were determined. On some of the samples (as shown in the tables) water-soluble phosphate was determined. On other samples phosphate soluble in 0.002 *N* $(NH_4)_2SO_4$ buffered to pH 4.0 was obtained by the same colorimetric method (6).

RESULTS OF NITRIFICATION STUDY

The data from the nitrification study are presented in table 1. The results represent the average of duplicate treatments.

In both the burned soils and for every treatment, nitrification started much better and continued more rapidly throughout the period of 12 weeks. The difference between the burned and the unburned is very marked. The data indicate that the supply of basic material is one important reason, though probably not the only one, for the difference. By comparing treatments 2 and 3 and 5 and 6, which are alike except for the addition of the basic material calcium carbonate, it is found that lime has increased nitrification very appreciably. On the burned soils the added lime approximately doubled nitrification as an average for the period, whereas on the unburned the increase was roughly 10-fold.

[3] The calcium carbonate will be called "lime" for convenience in the remainder of this paper.

The addition to the soil of litter alone or with ammonium sulfate did not have a very marked effect on nitrification by the burned soils. On the unburned soils the extra litter stimulated nitrification in every case. Gibbs and

TABLE 1

Nitrates as p.p.m. of nitrogen in the soil

SOIL USED	TREATMENT					
	1 Check	2 $(NH_4)_2SO_4$	3 Lime $(NH_4)_2SO_4$	4 Litter	5 Litter $(NH_4)_2SO_4$	6 Litter, Lime $(NH_4)_2SO_4$
Soil A, young reproduction:						
4 weeks	Trace	Trace	Trace	Trace	Trace	Trace
8 weeks	Trace	2.9	20	Trace	5.5	22.2
12 weeks	Trace	5.1	33.3	1.4	20.9	100.0
Totals	Trace	8.0	53.3	1.4	26.4	122.2
Average	Trace	2.7	17.8	0.5	8.8	41.7
Soil B, recent burn:						
4 weeks	7.2	5.3	28.8	7.6	3.3	26.1
8 weeks	30.9	28.6	110.0	33.3	27.7	110.0
12 weeks	36.5	61.7	100.0	30.6	51.5	100.0
Totals	74.6	95.3	238.8	71.5	82.5	236.1
Average	24.9	31.8	79.6	23.8	27.5	78.7
Soil C, old-timber:						
4 weeks	Trace	Trace	Trace	Trace	Trace	4.4
8 weeks	4.7	Trace	32	18.3	1.9	59.3
12 weeks	18.3	3.1	30	29.1	6.0	120.0
Totals	23.0	3.1	62	47.4	7.9	283.7
Average	7.7	1.0	21	15.8	2.6	61.2
Soil D, 2 year-old-burn:						
4 weeks	19.3	20.8	43.8	21.0	24.0	55
8 weeks	40.1	54.5	120.0	40.0	60.8	110
12 weeks	29.4	76.4	123.6	44.4	84.0	146
Totals	88.8	151.7	287.4	105.4	168.8	311
Averages	29.6	50.6	95.8	35.1	56.3	103.7
Average of "Burned over"	27.2	41.2	87.7	29.4	41.9	91.2
Average of "Unburned"	3.9	1.8	19.4	18.2	5.7	51.4
Average of all Soils	15.6	21.5	53.6	18.8	23.8	71.3

Werkman report that forest litter reduced nitrate production and that fern residues had but little inhibiting effect (3). The reason for the stimulation of nitrate production by the litter on these unburned soils is not known. Perhaps the litter may have resulted in ammonification, and the ammonia thus

produced served as basic material to stimulate nitrate formation. It is generally conceded that ammonia is produced rather abundantly in the humification of forest litter. Since tests for ammonia were not made, however, this cannot be verified.

The unburned soils were very slow in starting nitrification. This is true regardless of the addition of basic material. In the burned soils nitrification, as far as the data indicate, was about at a maximum at the end of 8 weeks. In the unburned soils nitrification was still improving at the end of 12 weeks and yet was far behind the burned soils in most cases. Evidently nitrifying organisms were favored by burning.

The most effective treatment for nitrification was a combination of litter, lime, and ammonium sulfate. The litter may introduce organisms, lime supplies base, and ammonium sulfate furnishes an easily nitrifiable form of nitrogen. With this combined treatment, after 12 weeks the unburned soils nitrify nearly as well as the burned.

Under natural conditions growth depends very largely upon the supply of nitrogen. Forest trees, however, may be able to use nitrogen in the ammonia form as well as in the form of nitrate. There must be a source of nitrogen, however, before ammonia can be produced. This source is principally the decomposing litter on the forest floor.

RESULTS OF STUDY OF SOLUBLE BASES

The effect of nitrification upon the solubility of soil bases is shown in table 2.

The data indicate that soluble calcium parallels in a general way the amount of nitrate produced. Of course the addition of lime has a bigger effect than any other treatment upon soluble calcium. However, the amount of soluble calcium is much higher in the burned soils than in the unburned where lime is not used. Since lime is an important ash constituent such a result may be expected. The addition of litter did not have much effect in increasing available calcium, as it likewise had little effect on nitrification.

The soluble potash is increased in most cases by the addition of ammonium sulfate. The addition of lime had little effect upon soluble potash. Soluble potash does not parallel the nitrates so closely as does the soluble calcium. Litter had almost no effect in increasing soluble potash. In the determination of soluble potash ammonia was eliminated by evaporation and burning.

The water-soluble phosphate is low and about the same in amount with all treatments, probably because of the strong phosphate-fixing power of these soils. However, burning liberates phosphate in the ash, and phosphate so liberated should become acid soluble. When the unburned and burned soils are compared there is consistently somewhat more acid-soluble phosphate in the latter. The largest amount of acid-soluble phosphate in the unburned soils was after the old burn, 61 p.p.m. Apparently nitrification has very little effect in increasing phosphate availability.

The data would indicate that regardless of how well trees may be able to

utilize the ammonia form of nitrogen, the process of nitrification should be favorable to good tree nutrition. The increased supply of available bases

TABLE 2

Water-soluble bases as p.p.m. of the ion of the soil

SOIL	NUTRIENTS*	TREATMENT					
		1 Check	2 $(NH_4)_2SO_4$	3 Lime $(NH_4)_2SO_4$	4 Litter	5 Litter $(NH_4)_2SO_4$	6 Litter, Lime $(NH_4)_2SO_4$
A. Young reproduction	Ca†	4.0	40.0	248.0	4.0	42.0	248.0
B. Recent burn	Ca	55.0	150.0	270.0	40.0	100.0	270.0
C. Old timber	Ca	13.0	Lost	.....	....	.....	.....
D. 2-year-old burn	Ca	25.0	150.7	271.2	37.5	158.1	331.5
Average	Ca	28.0	113.6	263.1	27.2	100.0	283.2
A Young reproduction	K‡	8.5	59.6	59.6	7.1	78.1	76.7
B. Recent burn	K	26.9	52.5	46.9	23.1	58.0	51.1
C. Old timber	K	26.9	39.8	55.4	39.8	52.5	52.5
D. 2-year-old burn	K	42.6	83.8	85.2	56.8	95.1	99.4
Average	K	26.2	58.9	61.8	31.7	70.9	70.0
A. Young reproduction	PO_4	0.23	0.23	0.23	0.23	0.23	0.23
B. Recent burn	PO_4	0.23	0.23	0.23	0.23	0.23	0.23
	ACID-SOLUBLE						
C. Old timber	PO_4	31.0	31.0	36.0	32.0	29.0	28.0
D. 2-year-old burn	PO_4	39.0	44.0	44.0	39.0	36.0	36.0
	LAST SAMPLING NITRATE						
A. Young reproduction	N	Trace	5.1	33.3	1.4	20.9	100.0
B. Recent burn	N	36.5	61.7	100.0	30.6	51.5	100.0
C. Old timber	N	18.3	3.1	30.0	29.1	6.0	120.0
D. 2-year-old burn	N	29.4	76.4	123.6	44.4	84.0	146.0
Average	Nitrate	21.2	36.6	71.7	26.4	40.6	116.5

Acid-soluble phosphate on soils A and B was 31 and 61 p.p.m. respectively.

* Nutrients are water-soluble unless otherwise indicated.

† Calcium was precipitated as oxalate and titrated with permanganate.

‡ Potash was determined by the cobaltinitrite method.

resulting from the nitrification process in a base-poor soil must be helpful. Any strong acid such as nitric or sulfuric produced by biological processes is

effective in dissolving bases. An equivalent amount of sulfuric acid is always liberated from the ammonium sulfate, when the ammonia is changed to nitric acid by nitrification.

GENERAL EFFECT OF BURNING

The forest tree depends for its nutrition largely upon the mineralization of the litter dropped upon the forest floor. Most of the humus material and the humification processes occur on top of the soil. As the process continues some of the soluble humus is leached into the topsoil, which is very porous and absorptive to a depth of 6 inches or more.

When fire enters the forest, both the litter and the humifying material are consumed at once. Even the humus in the immediate surface soil may be partly burned out. This is indicated in the data in table 3.

In the area burned over to dispose of slash, the humus content of the soil is low in the immediate surface and increases with depth. In the unburned area the humus content of the soil surface is high, and decreases with depth.

TABLE 3

*Humus content expressed as per cent of soil**

	DOUGLAS FIR UNBURNED PER CENT HUMUS	HEAVILY BURNED AREA PER CENT HUMUS
Surface inch	4.86	1.70
4½-inch to 7½-inch depth	4.81	3.78
10½-inch to 12½-inch depth	3.77	4.47

* Humus was determined by the modified Rather method (1).

These data of course do not take into consideration the actual litter and humus on top of the unburned soils.

The data of this work indicate that the immediate effect of burning is to produce a rather liberal supply of plant food. This is favorable both to soil organisms and to plants growing on the soil. Just after a burn which destroys the over growth there is likely to follow a rather profuse vegetation of wild grasses and herbs that produce excellent humus materials. Even legumes may volunteer. It is well known that clover starts easily when seeded in the ash after a burn. Leaving out of consideration damage that is done the trees, should the burn occur only once, or very infrequently, the effect upon soil fertility might not be very disastrous.

Nevertheless, what represents several years of leaf fall is entirely consumed by fire in a few minutes. Data collected by the senior author indicate that in this section in young forest there may be 10 tons or more of litter and humus material per acre on the forest floor. In old forests there are several times as much. More than 100 tons per acre has been reported (5).

In timber that is so dense as to prevent growth of grass, the renewal of so much humus material would require several years. The litter fall per season

probably does not exceed 2 tons and may be considerably less. Repeated burning, therefore, must soon result in a condition where there is no longer any organic materials in the soil to humify. Nature's method of renewing forest soils then becomes inoperative. Only one result can follow. Impoverished soils must lead to reduced tree growth, through lack of nutrients, reduced moisture capacity, and generally unfavorable conditions. It would seem rather dangerous to conclude, therefore, that because the immediate effect of burning is helpful the general practice even with controlled fires repeated frequently would be beneficial to the soil.

CONCLUSIONS

Nitrification in forest soils is stimulated by burning and the liberation of the basic ash materials.

Burning and the increased nitrification increase the soluble mineral nutrients in the soil, probably for some time after burning.

Burning destroys not only the organic matter on top of the soil, but may destroy some of that in the immediate soil surface.

The temporary effect of burning may be helpful at least in some respects, but, since the productivity of the forest soil depends upon gradual mineralization of the fallen litter, it does not appear reasonable to expect continuous and often repeated burning to improve forest soil fertility.

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INFLUENCE OF TEMPERATURE ON BACTERIAL NITRIFICATION IN TROPICAL COUNTRIES

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In previous communications (2, 3, 5) from these laboratories it has been stated that in tropical countries ammonification and nitrification in soil are mainly due to the photo-oxidation of amino acids to ammonia and of ammonium salts to nitrite and nitrate by the oxygen of the air. In order to throw more light on the mechanism of nitrification in soils of tropical countries, we have undertaken an investigation of the velocities of bacterial nitrification at various temperatures.

EXPERIMENTAL

A pure culture of nitrite-forming bacteria was prepared from garden soil by Omeliansky's method of elective culture, the medium employed being

Ammonium sulfate	2 to 2.5 gm.
Potassium phosphate	1 gm.
Magnesium sulfate	0.5 gm.
Sodium chloride	2 gm.
Ferrous sulfate	0.4 gm.
Distilled water	1,000 cc.
Magnesium carbonate	excess (0.5 gm. per 50 cc. of the medium)

Fifty cubic centimeter portions of this medium were taken out in 200-cc. Jena glass flasks, which were plugged with absorbent cotton and sterilized at 20 pounds pressure for 15 minutes. The ammonium sulfate was sterilized separately as a 10 per cent solution, and the required amount was added, with a sterile pipette, to the sterile flasks containing the other constituents of the medium.

One gram of the garden soil was inoculated into the flasks containing the culture medium and the flasks were then incubated at 30° to 35°. After a week, growth of the bacteria began and was indicated by the appearance of nitrite and disappearance of the ammonium salt. When all the ammonium salt was oxidized to nitrite, a fresh portion of ammonium sulfate was added. This second portion was oxidized much more rapidly, since the nitrite-forming bacteria had developed abundantly. After several additions of ammonium sulfate, a vigorous culture of the specific organisms was obtained. Now a drop of the suspension from the bottom of the flask was transferred to another flask

containing the same medium. After four or five such transfers, the culture obtained was fairly pure and rich enough in the specific organisms and was used for isolation purposes. In order to get the culture absolutely pure, a drop or two from the bottom was plated on a silica gel contained in a petri-dish, which was kept inverted in an incubator at 35°. The colonies developed after 15 days, and a single selected colony was inoculated into a fresh medium. The culture of the bacteria thus obtained was quite pure.

For the systematic study of bacterial nitrification, the following procedure was adopted. Various sets of experiments were thus arranged:

Solution A—10 per cent sterilized ammonium sulfite.
Solution B—containing other constituents of the medium in the proportions already stated.

Seventy cubic centimeters of solution B were taken in 250-cc. Jena glass conical flasks and 0.5 gm. magnesium or calcium carbonate added to it. The flask was then sterilized at 20 pounds pressure in an autoclave. Into each flask was introduced 2 cc. of the purified culture suspension after thorough shaking. The culture suspension was freed from traces of nitrite by repeated washing with sterilized distilled water. Two flasks under identical conditions were then kept in a thermostat at a definite temperature and the mean of the two results obtained with the two flasks was taken in order that the experimental error might be minimized. A control experiment under similar conditions but containing no bacteria was always conducted side by side at the same temperature. The amount of nitrite formed was estimated by a Duboscq colorimeter from time to time using a mixture of 0.8 per cent solution of sulfanilic acid in acetic acid of sp. gr. 1.04 and 0.5 per cent solution of α-naphthylamine in acetic acid of the same specific gravity as the indicator (colorimetric determination according to Griess-Ilsovay method). Equal amounts of the indicator were added both to the nitrite solution of the unknown strength and to the standard nitrite solution, and the color of the two was matched in the colorimeter. The amount of nitrite formation was taken to be directly proportional to the activity of the organisms. The experimental results obtained with $MgCO_3$ are recorded in table 1.

At 50° the amounts of nitrite formed in the flasks containing the bacteria were the same as in the control flasks containing no bacteria.

The results recorded in table 1 clearly show that the optimum temperature of nitrification with the tropical nitrite-forming bacteria is about 35°. The experiments of other workers, such as Omeliansky and Winogradsky, carried on in colder countries show that the optimum temperature is near 25° for the nitrite-forming bacteria. This clearly brings out the fact that the nitrite-forming bacteria possess the capacity of adapting themselves to changed surroundings. On account of the high temperatures existing for the greater part of the year in the tropics, the bacteria in soils of tropical countries have so modified themselves as to thrive best at 35°. It is interesting to note that

Panganiban (6), working in the Philippines, has also observed an optimum temperature of 35° with his nitrite-forming bacteria.

At temperatures lower than the optimum, a regular rising curve is obtained when the amount of nitrite formed is plotted against time. At the optimum temperature, where the activity of the bacteria is at the highest, nitrite is rapidly formed in the beginning and, as the concentration of the ammonium salt left is decreased, the velocity of nitrification is also diminished. In such cases, as is to be expected, an S-shaped curve is obtained.

Table 1 shows that at 0° the bacteria are capable of maintaining life activity for some days in the beginning, but a prolonged exposure to this temperature ultimately results in the complete stoppage of their life activity. Such a culture when brought back again to optimum conditions fails to show any further sign of growth even after being kept for 9 months. From these experiments we conclude that at 0° complete destruction of the bacteria occurs within

TABLE 1

Amount of nitrite formed at various temperatures

(Gm. of NO_2 per liter)

TIME	NITRITE FOUND AT TEMPERATURE OF						
	0°	15°	20°	25°	30°	35°	40°
hours							
67	0.000253	0.0001525	0.001169	0.005066	0.00792	0.008848	0.00064
115	0.000281	0.0002884	0.00175	0.008505	0.02304	0.0438	0.000689
163	0.000281	0.0004045	0.002779	0.01777	0.0497	0.09385	0.000844
187	0.000281	0.0006595	0.002953	0.02656	0.0673	0.1438	0.000950
211	0.000281	0.001318	0.00376	0.0386	0.08503	0.1987	0.001076
235	0.000281	0.002163	0.004468	0.05797	0.1026	0.2715	0.00141
259	0.000281	.....	0.00559	0.08585	0.12595	0.3585	0.001718

a few days. At 50° also we find that the bacteria are incapable of existence, as at this temperature our experiments gave equal amounts of nitrite formation both in the control flasks and in those containing the bacterial culture.

It has been reported by various investigators that calcium or magnesium carbonate is essential for the activity of the nitrifying bacteria. The function of the carbonate is twofold: firstly, it acts as a neutralizer of the acidity developed in the solution due to the oxidation of ammonium salts and thus maintains a constant pH value; and secondly, it supplies the organisms with their carbon requirements for the building up of their body.

Ashby (1) has found that nitrification proceeds more rapidly in the presence of magnesium carbonate than in the presence of calcium carbonate. This observation is also confirmed by S. Machida, who found nitrification to be favored more by magnesium carbonate than by calcium carbonate. In table 2 we are recording our results with both these carbonates.

These results show that the activity of our bacteria is higher in the presence

of calcium carbonate than of magnesium carbonate. Winogradsky has reported that an excess of free ammonia is detrimental to the growth and activity of nitrifying bacteria. We know that the medium becomes more alkaline in the presence of magnesium carbonate, which therefore liberates more ammonia, and hence the activity of the bacteria is considerably diminished. Calcium carbonate, on the other hand, does not impart so much alkalinity to the medium. This is probably due to the greater solubility of magnesium carbonate than of calcium carbonate. The smaller velocity of nitrification in the presence of magnesium carbonate may not be wholly due to increased alkalinity of the medium. From our experiments calcium carbonate appears to be about four to five times more efficient than magnesium carbonate. It appears that the calcium ions present in the system containing calcium carbonate may exert a specific action on the bacterial growth.

TABLE 2

Amount of nitrite formed at 30°

(NO_2 in gm. per liter)

TIME	NITRITE FOUND	
	With calcium carbonate	With magnesium carbonate
hours		
67	0.04379	0.00792
115	0.1178	0.02304
163	0.2084	0.0497
187	0.2649	0.0673
211	0.3415	0.08504
235	0.4326	0.1026
259	0.5075	0.1259

Solution A—3 cc. (10 per cent sterilized ammonium sulfate).
Solution *B*—70 cc. (containing other constituents of the specific medium and sterilized).
0.5 gm. magnesium or calcium carbonate.
Bacterial culture—2 cc. suspension.

Last summer, in the month of April, the soil temperature at Allahabad rose to 50° at 2 inches deep. May and June of 1933 were frequently cloudy, and the summer was a mild one.

Leather, working at Pusa, India, stated that the maximum temperature at Pusa may rise to 70° at the soil surface and 60° at a depth of 1 or 2 inches. In Egypt the recorded temperature is 65° at the surface and 56° at a depth of 2 inches. From our measurements on the influence of temperature on bacterial nitrification, we find that 35° is the optimum temperature for bacterial nitrification, which becomes practically negligible at 50°. Hence in tropics, nitrification in soil cannot be to any extent, of bacterial origin in the months of April, May, June, and July, when the soil temperature is much greater than the optimum for nitrification by bacteria. In these very months the amount of nitrate in soil is, however, the greatest (5). Even in colder countries, like

England and Germany, the soil temperature may rise to 35° or more in summer (8), although the optimum temperature for bacterial nitrification is stated to be 25°. In these countries bacterial nitrification must be greatly reduced in the summer months as a result of the soil temperature becoming much greater than the optimum temperature for bacterial nitrification, though the nitrate content of the soil in these countries is also largest in the summer time.

Dhar and Atma Ram (4) have shown that in the photo-oxidation of organic substances, specially of the amino acids and hydroxy compounds, appreciable amounts of formaldehyde are formed. The formaldehyde produced in the photo-oxidation of organic compounds present in the soil is likely to hinder the bacterial processes taking place in the soil, and hence the ammonification and nitrification in the soil due to bacteria will be curtailed by the presence of the formaldehyde, which is a powerful antiseptic and is produced in soil by the oxidation of the organic compounds by air in the presence of light.

In a private communication to one of the authors, Dr. A. S. Corbet, working at the Agricultural Research Station, Bracknell, Berkshire (England), makes the following statement: "From my experience in Malaya, where I was particularly struck by the paucity of soil microörganisms, I should think your results on photonitrification are of quite general application in tropical countries."

In the unmanured soil of the Institut de Brie-Comte-Robert, Winogradsky found only a few living organisms, three or four kinds of cocci, a few azotobacter, some mycelium of actinomyces, and a few yeast, but no bacilli or moulds. These are regarded by Winogradsky as the normal population of the soil. It is believed that in moist soils, most of the bacteria are concentrated in the first 2 feet of soil. The largest numbers are observed 1 to 3 inches below the surface. Because of the antiseptic properties of the solar radiations and the rapid evaporation of moisture from the soil surface, the number of bacteria is less on the surface. According to Waksman (9), soils under shade have the highest number of bacteria at a depth of 1 inch, and the numbers decrease with depth, whereas soils receiving sunlight contain the largest numbers of bacteria at a depth of 4 inches.

Recently it has been observed by us that dilute solutions of sodium or potassium nitrite are almost completely oxidized to nitrate when exposed to light along with solid substances like TiO_2, ZnO, Fe_2O_3, and sterilized soil in the absence of bacteria. In other words, from the experimental work carried on in these laboratories we find that not only are ammonium salts oxidized to nitrate by air in the presence of light but the nitrites are also, in their turn, readily converted into nitrate when exposed to light and air along with the photocatalysts. It seems, therefore, that the whole process of nitrification can be photochemical. Moreover it has been found by us that solutions of amino acids are readily oxidized to ammonia in the presence of air and light. Thus one of the important stages in the process of ammonification is also markedly accelerated by light.

The increase in fertility of soil as a result of exposure to sunlight as practiced in India, Egypt, and other countries from time immemorial can be easily explained from our observations. The increased production of ammonia and nitrate by partial sterilization of soil has been stated by authorities on agriculture to be not a necessary consequence of the higher number of the organisms present in the soil. As nitrification is essentially an oxidation reaction taking place on the soil surface, the various agencies: e.g., increase in the aeration, soil surface, and temperature up to a limiting value, will increase nitrification. Moreover sunlight or diffused light is always available and accelerates the formation of NH_3 from amino acids and the oxidation of NH_3 to nitrite and of nitrite to nitrate. It appears that the increase in fertility of soil on heating or exposure to sunlight, as is done by cultivators in many countries, is mainly due to increased oxidation of nitrogenous compounds, especially of the amino acids, causing the formation of NH_3 and to increased nitrification brought about by the soil becoming more porous and by the accentuation of the air supply and above all by the increased light absorption. When a soil is ploughed, fresh surfaces are exposed to sunlight and air and nitrification is facilitated.

It is generally agreed that nitrification is most active at the surface of the soil. According to Prescott and Piper (7) nearly 80 per cent of the nitrate accumulation takes place in the first $3\frac{1}{2}$ inches of the soil from the surface. From the observations recorded in this paper it seems to us that the soil temperature in the summer months in tropical countries must not be much below 50° even at the depth of $3\frac{1}{2}$ inches from the surface, although the bacterial nitrification has the optimum temperature of 35°. In Lyallpur, Punjab, in summer months, the soil temperature at 5 cm. depth is as high as 50° and at 30 cm. depth 37°. It appears, therefore, that in the first $3\frac{1}{2}$ inches of the soil, where there is maximum nitrification as observed by different investigators, very few bacteria can exist in tropical countries in summer months, although the amount of nitrate in soil is maximum in summer. We are led to conclude, therefore, that agencies other than bacterial must be active in causing nitrification, which is going on at a maximum speed in the soil in summer months within $3\frac{1}{2}$ inches of the soil. As we have observed that light markedly accelerates the oxidation of amino acids to NH_3, of ammonium salts to nitrites, and of nitrites to nitrates and that all these oxidations are accelerated by increase of temperature, we are led to believe that light absorption and increase of temperature play a more important rôle than bacteria in nitrification in summer months, when the nitrate content of the soil is known to be maximum in almost all countries.

In publications from these laboratories it has been shown that the oxidation of ammonium salts mixed with sterilized or unsterilized soil in covered vessels coated with Japan black enamel (i.e., receiving no light) when kept in the sun along with the vessels receiving light is much smaller than in those exposed to light. If nitrification were mainly a bacterial process the amount of nitrifica-

tion in the vessels in the dark with the unsterilized soil should not have been materially different from those exposed to light. We have made numerous comparative experiments like those on the oxidation of ammonium salts to nitrite and of nitrite to nitrate with both sterilized and unsterilized soil kept in light and in dark and we find that the formation of nitrite and nitrate is always much greater in the vessels exposed to light than in those kept in the dark.

From all the points discussed in this paper it appears to us that the process of nitrification in soil under ordinary conditions is due more to sunlight than to bacteria, especially in tropical countries.

SUMMARY

The optimum temperature of the nitrite formers in tropical soil is 35° as against 25° observed in the soil of temperate countries.

The velocity of bacterial nitrification is greater in the presence of calcium carbonate than in the presence of magnesium carbonate.

The soil temperature in tropical countries in summer markedly exceeds the optimum temperature for bacterial nitrification and may even be greater than the maximum temperature at which the nitrite-forming bacteria can exist. Hence in summer, nitrification in tropical soil cannot be mainly due to bacteria.

The amount of nitrate in soil is maximum in summer, and as most of the bacteria are likely to be killed by the high temperature prevailing in the soil, it is concluded that light plays an important rôle in nitrification in soil.

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MICROBIAL ACTIVITIES IN SOIL: I. NITROGEN FIXATION BY AZOTOBACTER AND ACTIVITY OF VARIOUS GROUPS OF MICROBES IN PALOUSE SILT LOAM[1]

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Under the prevailing system of alternate wheat and summer-fallow farming in the semiarid area of eastern Washington and other similar areas, both the stubble and straw are commonly left in the field. The extremely wide nitrogen-carbon ratio of this residue results in reduced yields of the succeeding crop, and therefore burning it in the field is customary previous to plowing for summer-fallow. Such a practice is conducive to rapid depletion of soil nitrogen and organic matter, which might be maintained or even increased if the straw residue could be used effectively in promoting non-symbiotic aerobic nitrogen fixation sufficiently to establish a more favorable nitrogen-carbon ratio during the process of decomposition. Inasmuch as moisture and temperature conditions in summer-fallowed soil are generally very favorable for microbial activity, it is of special interest to procure more definite information regarding the activity of Azotobacter and the effect of additions of carbonaceous organic residues upon their nitrogen fixing power in soils on which the production of wheat or other cereals is alternated with summer-fallow. The work here presented was designed chiefly to study the activity of these organisms and its relationship to that of other important groups of microbes in soils of different reactions and organic matter content to which additional supplies of carbonaceous organic material had been added.

METHODS OF PROCEDURE

A preliminary experiment was carried on to compare the nitrogen fixing power and activity of Azotobacter in a manured soil with that in a non-manured soil both of which had been used in a previous investigation (11). The amount of nitrogen fixed in these soils, both with and without applications of carbonaceous organic residue, was determined by analysis for total nitrogen at the beginning and the end of the experiment. The activity of Azotobacter was estimated at intervals by plating definite amounts of soil on silica gel plates, according to the method of Winogradsky (15). A more detailed study of the

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comparative activity of Azotobacter and several other groups of soil microbes in relation to the reaction and the nitrogen and organic matter content of variously treated soils was made subsequently. The numbers of bacteria, fungi, Azotobacter, and cellulose decomposing bacteria were determined as separate groups at frequent intervals, and periodical analyses were made for carbon dioxide, organic carbon, nitrate nitrogen, and total nitrogen. The experiments were conducted at room temperature which varied between 20° and 25° C.

Preparations of soil samples.—Soil samples representing the surface 8 inches of Palouse silt loam were obtained from two of a series of field plots used for soil experimental work during the previous 27 years, and from a 6-acre area of virgin soil covered with native grass vegetation which had never been disturbed by plowing or harvesting. One of the field plots had been cropped with winter wheat every year and had received an annual fall application of farm manure at the rate of 12 tons per acre. The other plot had been in wheat in odd years and in summer-fallow in even years and had received no other residue than the wheat stubble. Because of scarcity of rainfall during the growing season, the fields in cereal crops in this area usually are dried out to a depth of several feet for a period of two or three months in summer. Therefore, the soil samples were obtained late in October following the harvest of the wheat and after the first fall rains had thoroughly moistened the surface layer 10 to 12 inches deep. The moist soil was spread evenly on paper in the laboratory, allowed to dry at room temperature until it could be handled freely without danger of puddling, and passed several times through a 6-mesh screen to obtain a uniform mixture of the soil and to eliminate coarse roots and other coarse organic material. Portions equivalent to 1,500 gm. of dry soil were used for each experiment, and water was added until the moisture content and consistency of the soil were similar to those in the field when it is in the best condition for tillage operations. Under these conditions, which have proved to be very satisfactory for this work, the normal moisture capacity of the soil as expressed by Shaw (8) is closely approached. By the addition of water when necessary, this moisture content was maintained within narrow limits throughout the experiments.

Finely ground filter paper passed through a 60-mesh sieve was used as organic residue, and a sufficient amount of sodium nitrate was added to make the nitrogen content of the paper equivalent to 2.5 per cent. The amount of readily available nitrogen thus provided is in accordance with the findings of Sievers and Holtz (9), who estimated that organic residues applied to Palouse silt loam should contain from 2.25 to 2.50 per cent nitrogen to overcome the well-known temporary depression in accumulation of nitrate nitrogen following the addition of carbonaceous materials that are low in nitrogen. In the first two experiments 1 per cent calcium carbonate as well as filter paper was added to the soil to prevent the possible occurrence of restricted Azotobacter activity which is often ascribed to lime deficiency. The soil samples were thoroughly

mixed with the ingredients first by hand and finally by several passages through a 6-mesh screen before they were put into wide-mouth bottles in which they were packed by gently tapping the bottles on the table. The bottles were connected with an absorption train for carbon dioxide determinations.

Samplings for soil analysis.—Several cores, representing the full depth of the soil were obtained with a butter sampler from each bottle and thoroughly mixed. The amount of the sample required for analysis was weighed, and the excess returned to the bottles, which were shaken and gently tapped on the table to fill the holes made by the sampler and to pack the soil properly. Unless otherwise stated, samplings were made on the first and second day after the experiment was started and every two days for a week thereafter. Following this time the interval between samplings was gradually increased until it was seven days, beginning with the second or third week. The samples intended for microbial analysis were spread very thinly on clean paper and air dried at laboratory temperature immediately following sampling. In this manner air-dry soil was obtained in 30 to 45 minutes with a minimum delay in the determination of the various groups of organisms.

Microbial analysis.—Conn's (3) direct microscopic technic with minor modifications was adopted for determining the number of bacteria in the soil. The 0.015 per cent gelatin recommended by Conn for the preparation of the soil suspension was substituted by 0.1 per cent agar, and *erythrosin*, instead of *rose bengal*, was used as a staining fluid.

The number of fungi was determined from soil suspensions by the dilution plate method, using potato extract agar adjusted to pH 3.2 to 3.4 by addition of sterilized 0.1 *N* citric acid previous to preparing the petri plates. Duplicate plates were prepared for each dilution, and the numbers recorded were computed from average counts of duplicate plates.

The Azotobacter and the cellulose decomposing bacteria were grown on silica gel plates 15 cm. in diameter. Winogradsky's (14) nutrient solution was used for the Azotobacter plates, and Waksman's (12) nutrient solution in addition to a sheet of filter paper superimposed upon the silica gel served as a medium for the growth of cellulose decomposing bacteria. Duplicate plates were prepared, one receiving twice as much soil as the other. The air-dried, pulverized soil was spread evenly over the entire surface of the gel in the plate at rates varying between 100 and 500 mgm., the exact amount in each case being determined arbitrarily on the basis of the number of organisms present in the soil at the previous sampling. Average counts of duplicate plates served as a basis for calculating the number of organisms per gram of dry soil.

Chemical analysis.—Total nitrogen and nitrate nitrogen were determined by the Kjeldahl and phenol-disulfonic acid procedures respectively, according to the official and tentative methods of analysis (1). The carbon dioxide was determined according to the method proposed by Heck (7), and the total carbon by the wet combustion method of Friedemann and Kendall (5). The

pH values were obtained by means of the quinhydrone electrode in suspensions of one part of soil to five parts of water.

TABLE 1

*Nitrogen fixing power of unmanured and manured Palouse silt loam treated with lime and with or without 1 per cent filter paper**

DAY OF SAMPLING	N FIXED BY THE AZOTOBACTER IN 10 DAYS IN 500 MGM. AIR-DRY SOIL ON SILICA GEL PLATES			
	Unmanured soil		Manured soil	
	Lime + filter paper	Lime	Lime + filter paper	Lime
	mgm.	*mgm.*	*mgm.*	*mgm.*
1	2.52	1.26	12.04	9.52
2	3.36	1.54	8.68	8.96
3	2.80	0.28	7.84	9.24
4	2.52	0.84	6.58	9.10
5	1.82	2.38	10.36	7.28
6	2.52	3.78	7.70	8.96
7	2.88	1.12	9.52	9.24
8	1.96	1.68	9.52	8.68
10	1.84	1.26	8.68	7.84
12	1.64	1.12	7.28	8.84
14	1.96	2.12	7.92	7.42
17	2.10	1.96	8.96	9.24
20	2.26	2.10	7.84	8.52
24	1.96	1.84	9.32	8.96
27	1.84	1.26	8.84	7.28
31	2.38	1.82	8.96	8.68
34	1.92	1.96	9.52	9.10
38	0.70	0.56	9.96	9.52
45	1.26	1.12	8.84	8.26
52	1.54	1.68	9.10	7.70
66	0.96	0.84	8.96	9.24
73	1.12	1.64	9.52	8.84
80	1.96	1.82	8.68	9.26
87	1.88	1.12	7.70	8.84
94	1.26	0.98	9.32	8.52
101	1.16	0.84	8.16	7.96
108	0.96	1.36	9.12	8.84
115	1.12	1.14	8.76	8.18
Average.....	1.86	1.48	8.85	8.64

* $NaNO_3$ added to make the nitrogen content of the filter paper equivalent to 2.5 per cent.

EXPERIMENTAL RESULTS

The chief purpose of the preliminary experiment was to determine whether fixation of atmospheric nitrogen in amounts capable of measurement by analytical methods occurs in Palouse silt loam and whether a marked variation in the organic matter content of the soil results in any appreciable difference

in the amount of nitrogen fixed by Azotobacter. The data obtained when duplicate 1,500-gm. portions of the unmanured and manured samples were treated with calcium carbonate and further handled as outlined in the methods of procedure for this experiment are recorded in tables 1 and 2.

The results show clearly that there was a striking difference in both the activity of Azotobacter and the quantities of nitrogen fixed in the two soils. The activity of Azotobacter, as shown by the average values of nitrogen recovered from the silica gel plates, was approximately five times as great in the manured as in the unmanured soil, and the difference in the total amounts of nitrogen fixed in these two soils at the end of the experiment was even more outstanding. The gain in total nitrogen in the manured soil was remarkably large considering the relatively short period (115 days) in which this took place. Similar increases in nitrogen have been reported, however, by Emmert

TABLE 2

Nitrate nitrogen and total nitrogen of variously treated unmanured and manured Palouse silt loam at the beginning and at the end of the experiment

SOIL AND TREATMENT	BEGINNING OF EXPERIMENT			END OF EXPERIMENT		N FIXED, BASED ON 2,000,000 LBS. DRY SOIL
	pH	N as NO_3	Total N	N as NO_3	Total N	
		p.p.m.	*per cent*	*p.p.m.*	*per cent*	*lbs.*
Unmanured:						
Lime	6.9	5.4	0.1411	36.0	0.1442	62
Lime + filter paper*	6.9	....	0.1661	76.0	0.1679	36
Manured:						
Lime	7.0	17.5	0.2257	64.5	0.2604	694
Lime + filter paper*	7.0	....	0.2507	108.0	0.2978	942

* $NaNO_3$ added to make the nitrogen content of the filter paper equivalent to 2.5 per cent.

(4) under greenhouse conditions in soils supplied with large quantities of manure, and by Wilsdon and Ali (13) under field conditions in an arid to semiarid climate in soils poorly supplied with organic matter. The data in table 1 indicate that the activity of Azotobacter was stimulated when 1 per cent filter paper was added, and in table 2 it is shown also that the addition of filter paper to the manured soil resulted in increased amounts of fixed nitrogen, suggesting that fresh carbonaceous organic residue as well as the organic matter content of the soil has a direct or indirect influence upon the activity of Azotobacter.

In an attempt to gain more information relative to the effect of the soil organic matter content and of additions of organic residue on the activity of Azotobacter, the experiment was repeated with fresh samples, using essentially the same soil treatments but substituting for the unmanured soil a virgin soil in which the organic matter was derived entirely from the native grass vegeta-

tion, and intermediate in amount between that of the manured and unmanured soils. The method of procedure in this experiment was modified considerably. Instead of measuring the nitrogen fixing power of Azotobacter by spreading small quantities of soil on silica gel plates, the number of these organisms was determined. The total microbial activity and the comparative activity of the bacteria, fungi, and cellulose decomposing bacteria in relation to the activity of Azotobacter and as affected by soil organic matter and additions of carbonaceous organic residue were also determined. This was done at the intervals

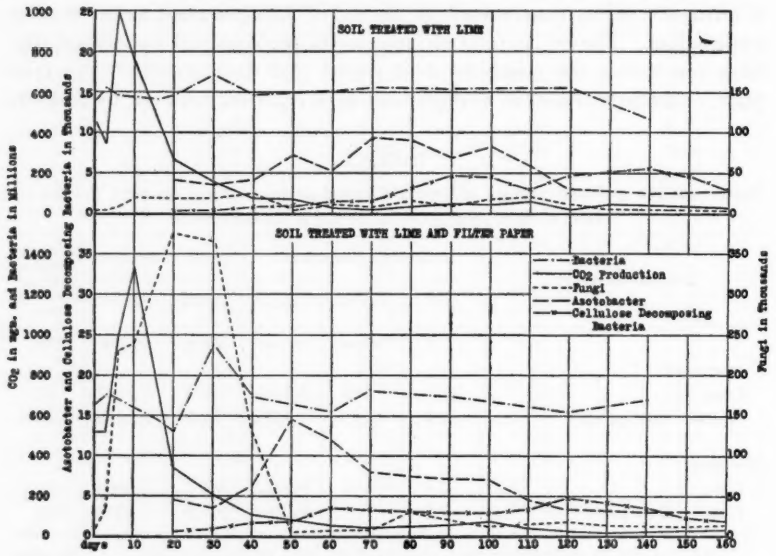

FIG. 1. MICROBIAL DEVELOPMENT AND CO_2 PRODUCTION IN MANURED PALOUSE SILT LOAM

Microbes given in numbers per gram and CO_2 in milligrams per 100 gm. of dry soil based on average values of determinations between 10-day intervals following first 10-day period. $NaNO_3$ added to make N content of filter paper equivalent to 2.5 per cent.

by the methods already described. The results are given in figures 1 and 2 and in tables 3 and 4.

Observations made while the work was in progress indicated that the reduction or increase in numbers of organisms of a specific group of microbes had a tendency to follow a somewhat gradual course, except for the first few days when marked changes in microbial activity occurred rapidly following the application of large amounts of fresh organic residue or the addition of the proper amount of moisture to air-dry soil. Therefore, the trend of changes taking place in the activity of these groups is represented in figures 1 and 2 as average values of two or three samplings during the first 10 days of the ex-

periment and as average values of the determinations made within periods of 10 days following that time.

Although the most intensive evolution of carbon dioxide occurred during the first 10 days, irrespective of soil or treatment, this activity was greatly stimulated by the addition of filter paper, which, however, was more effective in the virgin than in the manured soil. Following the initial 10-day period, the evolution of carbon dioxide proceeded at a low rate in all soils.

The maximum numbers of bacteria and fungi, which were the first to show increased activity, did not appear until several days after the peak in carbon

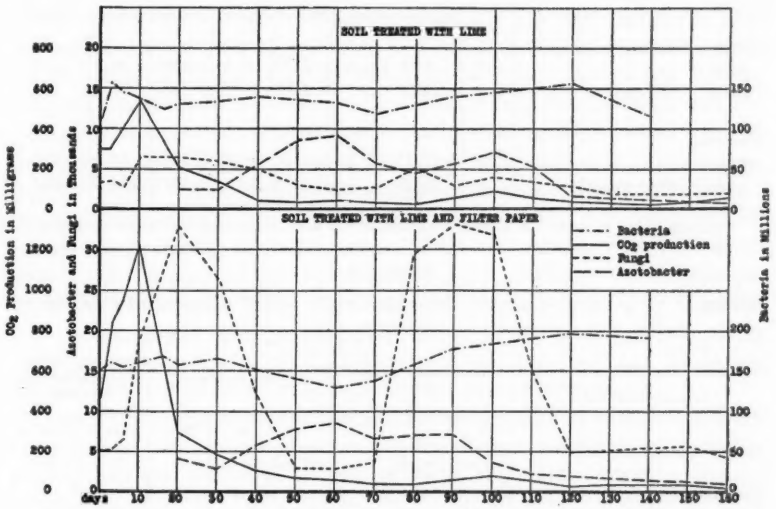

FIG. 2. MICROBIAL DEVELOPMENT AND CO_2 PRODUCTION IN VIRGIN PALOUSE SILT LOAM

Microbes given in numbers per gram and CO_2 in milligrams per 100 gm. of dry soil based on average values of determinations between 10-day intervals following first 10-day period. $NaNO_3$ added to make N content of filter paper equivalent to 2.5 per cent.

dioxide evolution had been reached. Thus, the activity of these organisms as shown by carbon dioxide evolution did not coincide with the increase in numbers of organisms, indicating that numbers alone are not necessarily an accurate index of the activity of soil microbes. The fact that bacteria and, especially, fungi were the first to take advantage of the available food supply suggests that they successfully competed with other groups of microbes in utilizing the readily available organic compounds. The fungi seemed to be more selective in their food requirements than the bacteria, as they responded quickly to the additions of organic residue, and the relative fluctuation in their numbers was much greater than in the numbers of bacteria, which remained

relatively uniform throughout the experiment. Although the activity of fungi was somewhat greater in the virgin than in the manured soils, the second

TABLE 3

Nitrate nitrogen and total nitrogen of variously treated virgin and manured Palouse silt loam at the beginning, at 80 days, and at the end of the experiment

SOIL AND TREATMENT	BEGINNING OF EXPERIMENT			80 DAYS		END OF EXPERIMENT		N FIXED, BASED ON 2,000,000 LBS. DRY SOIL AT END OF EXPERIMENT
	pH	N	N as NO_3	N	N as NO_3	N	N as NO_3	
		per cent	*p.p.m.*	*per cent*	*p.p.m.*	*per cent*	*p.p.m.*	*lbs.*
Virgin:								
Lime	6.9	0.2160	Trace	0.2255	9.6	0.2268	36.5	216
Lime + filter paper*	7.0	0.2410	....	0.2673	17.0	0.2687	94.0	554
Manured:								
Lime	7.0	0.2257	17.5	0.2716	19.4	0.2690	46.5	866
Lime + filter paper*	7.1	0.2507	....	0.2929	26.2	0.3039	154.0	1,064

* $NaNO_3$ added to make the nitrogen content of the filter paper equivalent to 2.5 per cent.

TABLE 4

Relation of nitrogen fixed to nitrogen and organic matter content of variously treated Palouse silt loam

SOIL AND TREATMENT	BEGINNING OF EXPERIMENT			END OF EXPERIMENT			N FIXED, BASED ON 2,000,000 LBS. DRY SOIL	
	N	C	N:C	N	C	N:C	Expt. 1	Expt. 2
	per cent	*per cent*		*per cent*	*per cent.*		*lbs.*	*lbs.*
Unmanured:								
Lime	0.1411	1.47	10.4	0.1442	1.41	9.8	62	...
Lime + filter paper*	0.1661	1.69	10.2	0.1679	1.52	9.1	36	...
Manured:								
Lime	0.2257	2.87	12.7	0.2604	2.83	10.5	694	...
Lime + filter paper*	0.2507	3.42	13.6	0.2978	3.11	10.4	942	...
Virgin:								
Lime	0.2160	2.25	10.4	0.2268	2.37	10.4	...	216
Lime + filter paper*	0.2410	2.48	10.3	0.2687	2.55	9.5	...	554
Manured:								
Lime	0.2257	2.87	12.7	0.2690	2.59	9.6	...	866
Lime filter paper*	0.2507	3.42	13.6	0.3039	3.34	11.0	...	1,064

* $NaNO_3$ added to make the nitrogen content of the filter paper equivalent to 2.5 per cent.

sudden rise in their number, occurring in the third month in the virgin soil treated with filter paper, can not be explained on the basis of the results obtained from the other soils.

The enumeration of Azotobacter and cellulose decomposing bacteria was not begun until the twentieth day, but judging from the trend of the curves it seems safe to assume that during the first month of the experiment their activity was not materially affected by any of the treatments. Following this period, and continuing for about 11 weeks, there was a marked increase in numbers of Azotobacter regardless of soil treatment, and after that time the numbers dropped to a low level in all cases. The increase in numbers following the first month corresponded closely with a decided stimulation in nitrogen fixation, for it was undoubtedly during this period that the greatest part of the gain in the total nitrogen was obtained, as can be noted from the data in table 3. However, the curves in figures 1 and 2 do not show that the activity of Azotobacter was materially affected by the addition of carbonaceous organic residue, whereas the data in table 3 and also in table 2 show clearly that the nitrogen fixation in the soils was greatly stimulated as a result of this treatment.

A possible symbiosis of Azotobacter with cellulose decomposing bacteria has been suggested by various investigators. The curves in figure 1 show that the numbers of cellulose decomposing bacteria were comparatively small and that their increased development began later but also subsided later than the increased activity of Azotobacter. They were relatively inactive at all times in the virgin soils, as their numbers seldom exceeded 150 colonies per gram of dry soil, and for that reason could not be shown conveniently in figure 2.

It would appear that on account of the relatively small numbers of the cellulose decomposing bacteria their activity did not have any significant bearing upon the activity and nitrogen fixing power of Azotobacter.

In considering the relationship of these various groups of microbes, it is obvious that in the presence of some available nitrogen the fungi and probably the bacteria also are quick in making use of the readily available organic compounds either originally present in the soil or supplied in the form of carbonaceous organic material. Supposedly the majority of these compounds are likewise an excellent source of energy for Azotobacter, but apparently they could not be used by this group as long as the fungi were in the competitive field. It is significant that the activity of Azotobacter became most intensive after this readily available food supply was exhausted and probably at a time when soluble nitrogen other than that supplied in the form of nitrate was in the process of accumulation, since it is well known that supplies of soluble nitrogen such as were added to the filter paper are used up in a few days when the microbial activity in the soil is optimum. It would seem, therefore, that the Azotobacter group thrives on compounds that are not readily available for fungi or possibly on dead cellular tissue of fungi and other microbes.

Winogradsky (16) and Ziemiecka (17) concluded, from their work on soils from experimental plots treated with various fertilizers over a period of years, that the addition of nitrogen fertilizers to the soil causes a reduction in the activity of Azotobacter presumably on account of the presence of available nitrogen. The data in table 3 show that nitrogen fixation was most active in

those soil samples that had received available nitrogen and that, at the time when nitrogen fixation was still active, contained larger amounts of nitrate nitrogen than were added annually to the field plots studied by the authors mentioned. The data in table 4 further indicate that the largest increase in total nitrogen occurred in the soil that was best supplied with organic matter and that the amounts fixed in the other soils were somewhat proportional to their organic matter contents. Apparently the quantities of soluble nitrogen that ordinarily accumulate in the soil under field conditions do not necessarily interfere appreciably with the normal functions of Azotobacter, provided the proper kind and amount of organic food supply are available. The nitrogen-carbon ratio of the soils did not seem to affect the functions of Azotobacter, as this ratio was approximately the same for the unmanured and virgin soils, whereas the nitrogen fixing power in these soils varied greatly.

The large gain in total nitrogen obtained in this experiment might appear to be unusual were it not for the fact that the manured soil, which was also used in the preliminary experiment and received identical treatments, gave strikingly similar results. It is significant that by far the largest part of the increase in total nitrogen was accumulated during the short time in which the Azotobacter group was very active and under conditions of moisture and aeration that were unfavorable for anaerobic organisms. At the same time, it should be noted that in three of the four samples receiving filter paper the process of nitrogen fixation was favored decidedly by the addition of fresh carbonaceous organic residue, although the activity of Azotobacter as shown by numbers present did not appear to be affected correspondingly. Possibly the observed increase in nitrogen cannot be fully accounted for by the activity of the Azotobacter group. Carter and Greaves (2) and Greaves and Greaves (6) have isolated a number of actinomyces and bacteria that seem to be capable of fixing small amounts of atmospheric nitrogen. This phase of the problem is worthy of further investigation. It is possible also that the remarkable fixation observed in this experiment would rarely, if ever, occur under field conditions or might not be reproduced in the laboratory if the soil samples were obtained from the field at a different time of the year. In previous work in this laboratory (10) it was shown that the number of active Azotobacter fluctuates greatly during different seasons of the year. That the activity of these organisms in the field may also vary from year to year was demonstrated by the work of Wilsdon and Ali (13), who found large increases in total nitrogen in certain soils during one year and no appreciable gains during the three following years. The activity of the Azotobacter group under field conditions seems to be erratic, and much additional work will be required to understand properly its relationship to different soil and environmental conditions.

The results of the two experiments with Palouse silt loam show conclusively that non-symbiotic aerobic nitrogen fixation was stimulated when large quantities of carbonaceous organic material were applied to those soils that were best supplied with organic matter and, furthermore, the data are strongly

suggestive that in the utilization of fresh carbonaceous organic residue there is a sequence of activity of important groups of microbes which definitely affects the process of non-symbiotic aerobic nitrogen fixation. Should these observations be borne out by future studies, the huge quantities of straw residue now commonly destroyed by fire in the wheat fields of semiarid areas could be returned to the soil profitably, since this residue could be made to serve not only as a valuable source of energy for the process of atmospheric nitrogen fixation, but also as an indispensable foundation for the maintenance of the rapidly disappearing soil organic matter. Further work on this problem is in progress and will be reported in a subsequent paper.

EFFECT OF SOIL REACTION ON MICROBIAL ACTIVITY

The extraordinary response in development of fungi, following the addition of filter paper to soils of neutral reaction which is generally presumed not to be favorable for the growth of fungi, suggests that the food supply may be a more important factor in the activity of these and other soil microbes than has been suspected heretofore. In order to study the effect of soil reaction on the total microbial activity and on the activity of specific groups of microbes, three portions each equivalent to 1,500 gm. of dry soil of the manured and unmanured samples respectively were adjusted to different reactions. On account of the high buffer capacity of Palouse silt loam, large quantities of acid or basic substances were required to bring about the desired changes in hydrogen-ion concentration. Since the addition of a large quantity of a single acid, such as sulfuric acid, for example, may have specific effects other than its effect on reaction, a mixture of equivalent parts of sulfuric, citric, and hydrochloric acids was used to overcome this difficulty, at least partially. Calcium carbonate was used as basic material to obtain the desired alkalinity of the alkaline soils.

The reaction of the acid samples was adjusted to a value of pH 5.5, that of the alkaline samples to a value of pH 8.1, and that of the two remaining samples was kept at the original hydrogen-ion concentration of pH 7.0. These values were maintained in the moist samples for 8 days before the 1 per cent finely ground filter paper treated with sodium nitrate as described in the methods of procedure was applied. The microbial activity was then determined in the same manner as in the previous experiment, except that the interval between samplings was 5 days for the first 3 weeks, and 10 days for the remaining period.

There was no appreciable change in reaction of the soils 3 weeks after the experiment was in progress, but, as can be seen from the data in table 5, a considerable change in pH value had occurred in the acid and alkaline samples at the end of the experiment. The change in both the acid and alkaline samples had proceeded in the direction of neutrality, probably a result of the buffer effect of the soils and not of the microbial activity.

The total microbial activity as shown by the carbon dioxide curves in figure

3 was slightly greater in the acid than in the neutral or alkaline soils during the first 10 days, but after that time it was very similar in all soils. The data in table 5 show also that the total amounts of carbon dioxide liberated from the soils during the 3 months in which the experiment was in progress were greatest in the acid soils. The differences may not be significant, as the organic acid, which was added to bring about the desired acidity, probably supplied sufficient readily available food to account for the apparent increased liberation of carbon dioxide.

The activity of the bacteria as indicated in figure 3 was comparatively uniform in all soils regardless of hydrogen-ion concentration. The activity of the Azotobacter and cellulose decomposing bacteria, as shown in the same figure, varied somewhat in the different soil samples; but these variations were not consistent and, therefore, cannot be considered significant. The activity of fungi, however, was markedly affected by soil reactions.

TABLE 5

*Moisture content, pH value, and CO_2 production in unmanured and manured Palouse silt loam adjusted to distinctly acid, neutral, and alkaline reactions and treated with 1 per cent filter paper**

	UNMANURED SAMPLES			MANURED SAMPLES		
	1	2	3	1	2	3
Per cent moisture at beginning	19.50	19.50	19.50	22.00	22.00	22.00
Per cent moisture at end	19.50	19.30	19.50	22.00	21.00	22.00
pH at beginning	5.5	8.1	6.9	5.5	8.1	7.0
pH at end	6.1	8.2	6.9	6.3	7.8	7.2
Total CO_2 in gm. evolved from 100 gm. of soil	1.51	1.33	1.24	1.46	1.42	1.37

* $NaNO_3$ added to make the nitrogen content of the filter paper equivalent to 2.5 per cent.

The effect of alkalinity on the development of fungi in the soil adjusted to a value of pH 8.1 is obvious, as the numbers were much smaller than in either the neutral or acid soils. This may have an important bearing upon the microbial activity of soils of arid regions where distinctly alkaline or alkali soils prevail, but is applicable only in exceptional cases in soils of the humid regions. In this connection it is significant to note that the activity of this group of microbes in the distinctly acid soils with a pH value of 5.5 did not vary greatly from that in the neutral soils with a pH of 7.0. Perhaps a more acid reaction than pH 5.5 might have been more favorable for the fungus group, but that is not so important from the practical standpoint, as the range of hydrogen-ion concentrations of the majority of soils under cultivation falls within the extreme pH values covered by this experiment. It might be expected also that other soils with the same reactions as the ones used in this experiment, but with different inherent characteristics, would give different results.

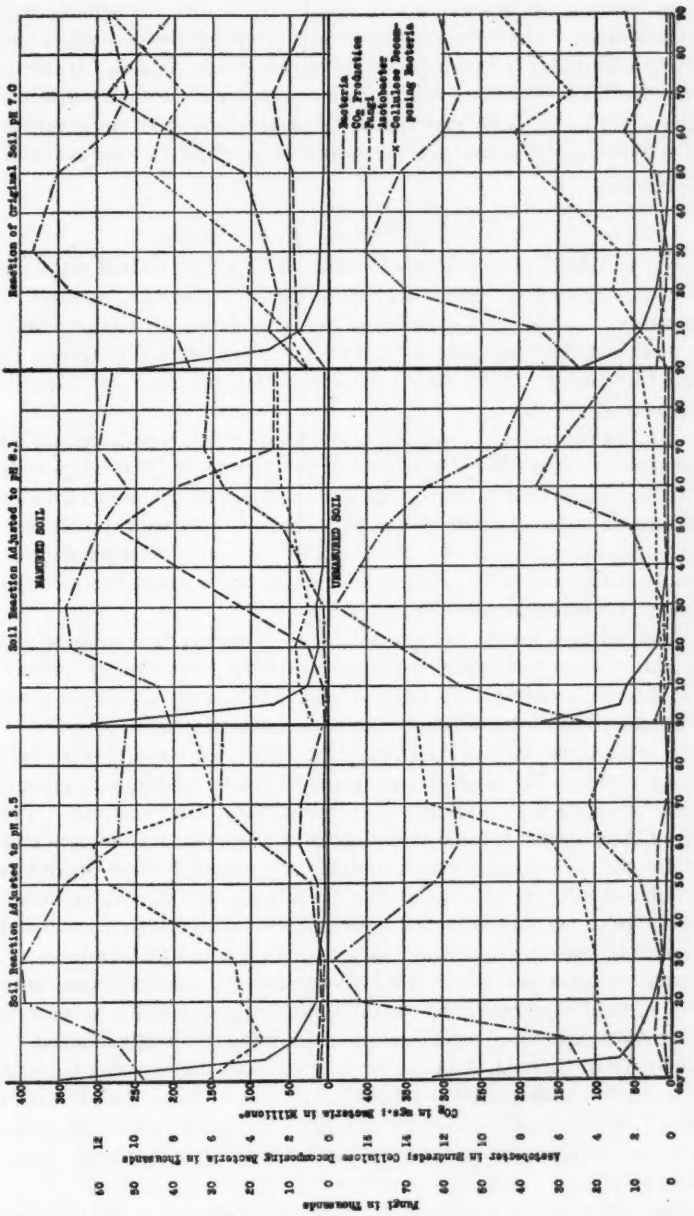

FIG. 3. MICROBIAL DEVELOPMENT AND CO_2 PRODUCTION IN MANURED AND UNMANURED PALOUSE SILT LOAM AT DIFFERENT pH VALUES

Microbes given in numbers per gram and CO_2 in milligrams per 100 gm. of dry soil based on average values of determinations between 10-day intervals.

The significant point brought out by these data is that the activity and development of several important groups of microbes proceeded normally under a relatively wide range of hydrogen-ion concentrations in the soil. It seems, therefore, that the hydrogen-ion concentration as such, provided it is within the limits occurring in the large majority of cultivated soils, does not affect the normal activity of the general soil microflora so much as does the available food supply.

SUMMARY

Nitrogen fixation in three different Palouse silt loam soils treated with lime and carbonaceous organic residue was determined in 1,500-gm. samples in the laboratory. A study was also made of the comparative activity of Azotobacter, total bacteria, fungi, and cellulose decomposing bacteria in these soils as affected by added carbonaceous organic material, reaction, and nitrogen and organic matter content.

The quantities of nitrogen fixed during the course of the experiment varied with the organic matter content of the soils. On the basis of 2,000,000 pounds of dry soil the gain in total nitrogen ranged from 36 pounds for the soil with an original organic carbon content of 1.47 per cent to 1,064 pounds for the soil with an original organic carbon content of 2.87 per cent. Nitrogen fixation was greatly stimulated in three of the four soil samples which had received large amounts of carbonaceous organic residue.

The total nitrogen content of the soil, the nitrogen-carbon ratio of the organic matter, or the comparatively large quantities of nitrate nitrogen present in the soils while atmospheric nitrogen fixation was in progress, did not seem to affect the activity of Azotobacter to any appreciable extent.

In the presence of available nitrogen, the fungi and bacteria were first to make use of the readily available organic compounds either originally present in the soil or supplied by additions of carbonaceous organic material. The activity of Azotobacter became most intensive after this readily available supply of organic compounds was exhausted, and apparently these organisms were capable of utilizing compounds that are not readily available for fungi.

The activity of cellulose decomposing bacteria was comparatively small and did not seem to have any significant bearing upon the activity of Azotobacter.

The transformation of organic residue was associated with a distinct sequence in development of important groups of soil microbes through which the process of non-symbiotic aerobic nitrogen fixation appeared to be directly affected.

The activity of bacteria, Azotobacter, and cellulose decomposing bacteria was not materially affected by soil reactions ranging between the pH values of 5.5 to 8.1, whereas the development of fungi was greatly retarded when the hydrogen-ion concentration was at pH 8.1 and slightly favored when it was at pH 5.5.

The apparently normal activity of these important groups of soil microbes in a wide range of soil reactions suggests that the available food supply and

possibly also the nature of the inherent soil characteristics are more potent factors in the activity of the soil microflora than is soil reaction, provided this reaction is within the limits generally occurring in the large majority of cultivated soils.

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PHYSIOLOGICAL STUDIES ON RHIZOBIUM: II. THE EFFECT OF NITROGEN SOURCE ON OXYGEN CONSUMPTION BY RH. MELILOTI, RH. TRIFOLII, AND RH. PHASEOLI[1]

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In the first paper of this series (1), data were presented showing the effects of nitrogen source on oxygen consumption by *Rhizobium leguminosarum*. In that work it was shown that in the absence of combined nitrogen there was no stimulation of growth, and that the cells continued to respire at a slightly decreasing rate throughout the duration of the tests. When yeast extract was added to the medium, there was a marked stimulation in the rate of oxygen consumption, the increase in rate being somewhat proportional to the amount of yeast extract added. After a certain period, usually about 12 hours, the rate of oxygen consumption became practically uniform, and presumably the cells, at that time, had passed their logarithmic phase of growth and from then on were in a resting state, but they continued to respire. When other nitrogenous materials—ammonium chloride, sodium nitrate, urea, or alanine—were added to the medium instead of yeast extract, there was no marked stimulation of growth irrespective of the kind or amount added, within the limits of the experiment. There was some indication that the ammonium chloride and urea, in the highest concentrations, slightly depressed oxygen consumption. It is not definitely known whether it was the nitrogen or some other constituent of the yeast extract that was the active agent in stimulating oxygen consumption and growth.

In order to secure further information concerning the nitrogen nutrition and metabolism of this important and interesting genus of bacteria, further experiments, similar to those already conducted with *Rh. leguminosarum* have been planned in which the effects of the various nitrogen sources on the consumption of oxygen by other species of the genus have been investigated. In the work reported here, studies were made with *Rh. trifolii*, *Rh. meliloti*, and *Rh. phaseoli*, the root-nodule bacteria of red clover, alfalfa, and garden beans, respectively.

The organisms were grown in a medium to which no combined nitrogen was added and also in media containing nitrogen from different sources. The nitrogen sources employed in this work were bacto-yeast extract, ammonium

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chloride, sodium nitrate, urea, and alanine. To separate cultures, these materials were added in various amounts to give the following concentrations of nitrogen: 0.72, 7.2, 36.0, 72.0, and 144.0 p.p.m. of solution.

The oxygen consumed by the organisms in the various media was measured in respiration vessels and micromanometers of the type used by Warburg (2) in his studies on the metabolism of tumors. The details of the procedure and also the methods followed in preparing the inocula are described in a previous paper (1).

RESULTS

The results obtained in the studies with the different species under consideration are presented separately in the following.

TABLE 1

Oxygen consumption by Rhizobium trifolii in media containing yeast extract

TIME	NO NITROGEN	AMOUNTS OF NITROGEN AS YEAST EXTRACT IN MEDIA				
		0.72 p.p.m.	7.2 p.p.m.	36.0 p.p.m.	72.0 p.p.m.	144.0 p.p.m.
hrs.	*c.mm.*	*c.mm.*	*c.mm.*	*c.mm.*	*c.mm.*	*c.mm.*
0	0.0	0.0	0.0	0.0	0.0	0.0
2	8.6	12.6	14.8	19.8	19.1	19.0
4	20.9	30.5	36.0	45.3	45.6	43.4
5	33.2	45.4	52.3	63.7	73.7	62.0
6	35.0	47.6	60.1	76.4	76.2	73.8
8	44.0	57.3	80.9	76.2	116.4	110.5
10	52.6	66.2	101.4	146.4	170.9	167.6
12	54.2	68.1	112.7	173.7	224.1	241.0
14	61.0	75.5	129.7	216.2	288.4	339.7
16	69.0	84.4	148.8	259.4	358.2	443.4
18	74.0	90.3	162.9	302.6	425.2	546.6
20	83.5	102.9	183.0	379.8	501.3	656.3
22	92.8	112.9	199.6	428.6	576.8	762.2
24	100.0	121.8	215.2	476.4	651.3	863.8
26	108.0	132.5	232.9	525.2	724.8	959.2
28	114.4	140.7	245.9	568.4	797.0	1,046.8
30	120.8	149.2	260.7	609.4	876.5	1,130.0

Rhizobium trifolii.—The data obtained in the oxygen consumption experiments with *Rh. trifolii* when grown in media containing varying amounts of bacto-yeast-extract are shown in table 1. The data show that throughout the entire test the cells in the no-nitrogen medium continued to respire and consume oxygen. There may have been some slight growth and multiplication, by the organisms, during the first 3 or 4 hours after they were introduced into the medium. If there was any growth at that time it was undoubtedly induced by the small amount of nitrogen carried over in the inoculum. The exact amount of nitrogen introduced in this way was not known, but it is evident

that it was extremely small, as considerable care was taken in the preparation of the inoculum to have it as free of nitrogen as possible. After the fifth hour the rate of oxygen consumption was at a minimum, and it continued at this low point throughout the test period. Undoubtedly the cells were in a state of rest during that time, and there was no growth or multiplication.

In the medium containing 0.72 p.p.m. of yeast extract nitrogen there was some stimulation of growth in the early period, but it may be clearly seen that the increased rate of oxygen consumption did not continue long, presumably because the small amount of yeast extract in the medium was soon used by the organisms. After the period of stimulation, respiration and oxygen consumption continued at a fairly uniform rate similar to that in the medium

TABLE 2

Oxygen consumption by Rhizobium trifolii in media containing ammonium chloride

TIME	NO NITROGEN	AMOUNTS OF NITROGEN AS AMMONIUM CHLORIDE IN MEDIA				
		0.72 p.p.m.	7.2 p.p.m.	36.0 p.p.m.	72.0 p.p.m.	144.0 p.p.m.
hrs.	*c.mm.*	*c.mm.*	*c.mm.*	*c.mm.*	*c.mm.*	*c.mm.*
0	0.0	0.0	0.0	0.0	0.0	0.0
2	10.7	11.7	9.3	12.0	9.5	11.1
4	16.8	16.6	14.0	15.3	13.1	13.4
6	24.7	22.6	19.7	20.0	18.0	16.3
8	34.8	30.6	26.5	25.7	23.0	20.0
10	44.3	38.8	33.9	34.0	30.4	24.1
12	52.6	47.0	41.8	40.8	39.3	28.3
15	64.6	60.3	53.4	54.9	50.8	34.0
18	75.6	74.9	65.3	67.2	62.5	39.3
20	84.1	86.4	76.3	76.5	72.0	43.8
22	92.0	97.8	86.1	83.4	80.0	47.2
24	98.4	109.6	97.8	87.9	88.7	50.9
26	104.9	119.3	107.4	96.8	96.1	53.6
28	110.5	130.1	116.6	108.1	103.1	56.7
30	118.9	144.4	138.5	112.6	112.5	60.1

without nitrogen. The total amount of oxygen consumed by the organisms in the yeast extract medium during the test was higher than that consumed by the organisms in the medium without yeast extract. This was undoubtedly due to the slightly greater stimulation in the early part of the test.

In the media containing the larger amounts of yeast extract, there was also a stimulation of growth in the early part of the test. The larger the amount of yeast-extract in the medium the greater was the stimulation of growth and oxygen consumption. The increase in rate of oxygen consumption lasted for a period of about 14 hours, during which time the organisms were undoubtedly growing rapidly and they were, presumably, in the logarithmic phase of growth. After that period the rate of oxygen consumption remained nearly uniform, except for a few unaccountable variations, until the end of the test. During

this time the organisms probably had passed into the later phases of the growth cycle and presumably they were in a resting condition, but they continued to respire and consume oxygen.

TABLE 3

Oxygen consumption by Rhizobium trifolii in media containing sodium nitrate

TIME	NO NITROGEN	AMOUNTS OF NITROGEN AS SODIUM NITRATE IN MEDIA				
		0.72 p.p.m.	7.2 p.p.m.	36.0 p.p.m.	72.0 p.p.m.	144.0 p.p.m.
hrs.	*c.mm.*	*c.mm.*	*c.mm.*	*c.mm.*	*c.mm.*	*c.mm.*
0	0.0	0.0	0.0	0.0	0.0	0.0
2	2.7	3.7	1.8	7.8	3.5	7.1
4	4.6	5.9	4.6	11.3	6.3	9.1
6	7.1	8.4	6.7	15.5	8.4	11.1
8	11.8	12.0	9.9	20.2	12.3	14.9
10	15.4	15.9	14.0	26.4	17.0	18.8
12	21.0	19.5	17.0	31.5	19.9	22.1
14	27.4	23.5	22.7	37.2	25.5	27.8
16	33.0	27.5	26.5	43.1	30.4	32.6
18	37.7	31.7	30.5	48.0	33.9	35.2
20	42.9	37.2	36.2	56.1	39.8	41.7
22	47.8	42.4	40.7	62.5	44.3	46.0
24	51.7	48.1	45.6	68.4	49.2	50.1
26	56.6	54.0	51.3	76.2	54.8	55.5
28	61.0	58.2	54.5	83.0	59.1	58.4
30	66.1	64.0	60.7	92.0	66.2	64.6

TABLE 4

Oxygen consumption by Rhizobium trifolii in media containing urea

TIME	NO NITROGEN	AMOUNTS OF NITROGEN AS UREA IN MEDIA				
		0.72 p.p.m.	7.2 p.p.m.	36.0 p.p.m.	72.0 p.p.m.	144.0 p.p.m.
hrs.	*c.mm.*	*c.mm.*	*c.mm.*	*c.mm.*	*c.mm.*	*c.mm.*
0	0.0	0.0	0.0	0.0	0.0	0.0
2	7.6	8.0	9.6	9.2	3.5	8.8
4	13.0	11.9	13.6	12.5	7.8	14.0
6	18.1	17.1	18.7	17.3	12.7	18.2
9	26.9	27.5	27.3	25.2	20.7	25.9
12	36.8	40.1	37.6	36.5	31.9	37.0
15	43.0	49.0	46.1	45.0	40.3	45.2
18	54.7	62.2	62.4	59.9	55.4	59.2
21	64.6	71.1	78.7	75.5	73.1	74.4
23	70.5	80.2	91.4	84.0	77.7	79.2
26	78.9	90.3	106.7	98.6	92.4	90.5
28	83.5	96.2	117.6	109.2	102.2	97.3
30	88.7	101.7	130.3	119.0	110.9	104.5

In the media containing the other forms of nitrogen, ammonium chloride, sodium nitrate, urea, and alanine, there was no large stimulation of oxygen

consumption such as there was in the yeast extract media. This is shown by the data for the consumption of oxygen by *Rh. trifolii* in these media which are recorded in tables 2, 3, 4, and 5. The data indicate that small amounts of nitrogen as ammonium chloride probably stimulated oxygen consumption and growth to a very slight extent, but larger amounts seemed to be definitely depressive.

The nitrogen added as sodium nitrate seemed to have no effect on the rate or amount of oxygen consumed, irrespective of the amount added. The nitrogen added as urea probably was stimulative to a very limited extent, but the nitrogen as alanine was definitely stimulative, although the extent of the stimulation was not great in any case when compared with the stimulation

TABLE 5

Oxygen consumption by Rhizobium trifolii in media containing alanine

TIME	NO NITROGEN	AMOUNTS OF NITROGEN AS ALANINE IN MEDIA				
		0.72 p.p.m.	7.2 p.p.m.	36.0 p.p.m.	72.0 p.p.m.	144.0 p.p.m.
hrs.	*c.mm.*	*c.mm.*	*c.mm.*	*c.mm.*	*c.mm.*	*c.mm.*
0	0.0	0.0	0.0	0.0	0.0	0.0
2	12.6	17.4	15.8	14.0	8.2	14.8
4	19.4	25.6	23.6	21.1	15.2	23.0
6	26.2	35.3	33.5	28.9	22.2	30.5
8	34.8	48.7	45.9	39.5	32.0	41.0
10	42.0	60.3	56.5	50.5	42.0	52.0
13	54.6	82.4	78.0	69.9	60.6	71.3
15	63.2	95.8	91.4	83.3	73.9	85.6
18	74.5	111.4	110.5	102.4	91.4	104.6
21	87.8	130.0	134.5	127.4	114.4	127.0
23	95.8	141.2	149.3	143.4	127.7	140.6
26	106.9	156.4	170.1	172.8	148.0	158.3
28	114.3	167.3	185.4	196.8	161.3	171.2
30	122.1	176.5	201.7	220.4	174.8	184.1

induced by the yeast extract. The comparative effects of the different forms of nitrogen on the consumption of oxygen by *Rh. trifolii* are shown in figure 1. The curves in this figure represent the ratios of the amounts of oxygen consumed by the organisms in the various media compared with the amount consumed in the medium without added nitrogen. It is shown that, in general, the respiration and growth of *Rh. trifolii* were affected in the same manner by the various forms of nitrogen as were the respiration and growth of *Rh. leguminosarum* which were considered in a previous paper (1).

Rhizobium meliloti.—The amounts of oxygen consumed by *Rh. meliloti* when grown in the media containing yeast extract in various concentrations are shown graphically in figure 2. Although smaller amounts of oxygen were consumed by this organism than by *Rh. trifolii* when grown in yeast extract media, the same general effects were exhibited. There was little or no growth

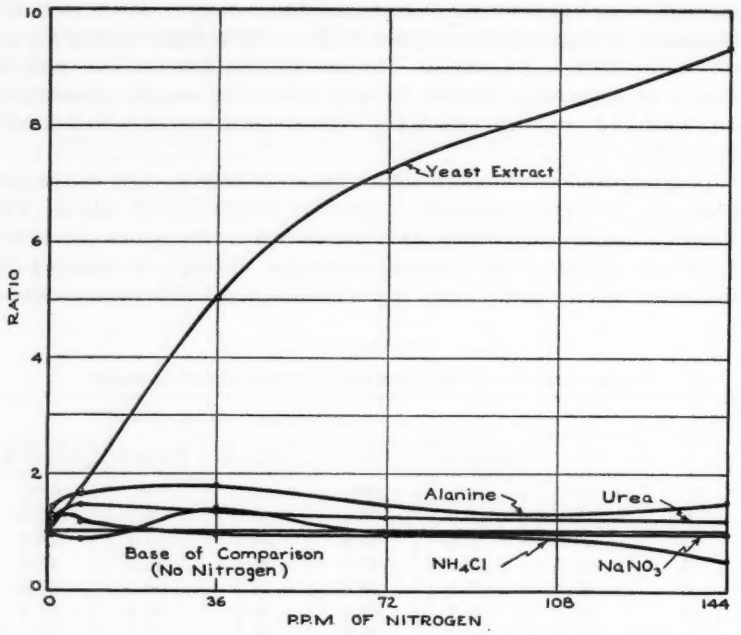

FIG. 1. THE RATIOS OF THE AMOUNTS OF OXYGEN CONSUMED BY *Rhizobium trifolii* IN THE VARIOUS NITROGENOUS MEDIA TO THAT CONSUMED IN THE MEDIUM WITHOUT NITROGEN

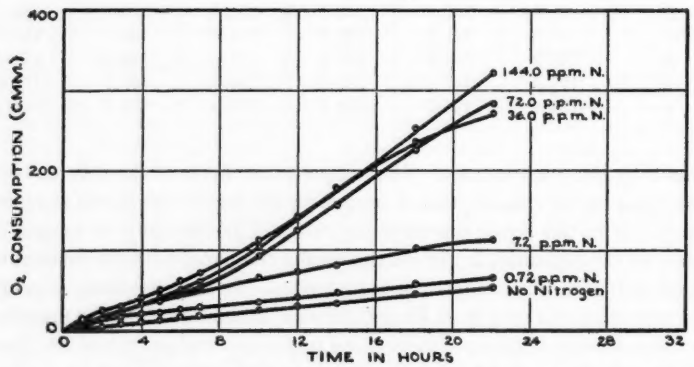

FIG. 2. OXYGEN CONSUMPTION BY *Rhizobium meliloti* IN MEDIA CONTAINING YEAST EXTRACT

in the medium without combined nitrogen, but the organisms continued to respire and consume oxygen at a fairly uniform rate throughout the entire period of study. On the other hand, the yeast extract definitely stimulated

growth, and increased the rate of respiration at least where the larger amounts were present, for a period of about 12 to 14 hours. The rate of increase, however, did not appear to be quite so great as in the case of *Rh. trifolii*. As was the case with the other species, after the organisms passed the logarithmic period of growth, the rate of oxygen consumption became fairly uniform or decreased slightly, indicating that the organisms were in the resting state, and probably that some were dead. It is also quite probable that no growth was taking place during this period.

When *Rh. meliloti* was grown in media containing the other forms of nitrogen, entirely different results were obtained from those in the experiments with *Rh. leguminosarum* and *Rh. trifolii*. The organisms of this species were definitely stimulated by ammonium chloride, sodium nitrate, urea, and ala-

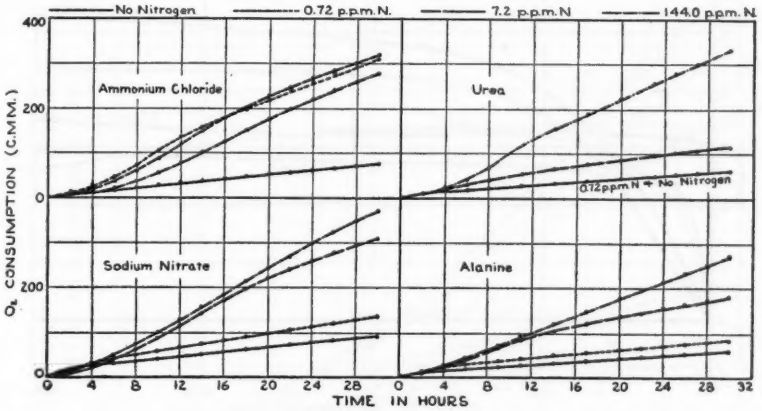

FIG. 3. OXYGEN CONSUMPTION BY *Rhizobium meliloti* IN MEDIA CONTAINING AMMONIUM CHLORIDE, SODIUM CHLORIDE, UREA, AND ALANINE

nine, and considerably larger amounts of oxygen were consumed during the period of the test. This is shown by the curves in figure 3.

There was a definite and rapid increase in the rate of oxygen consumption in the medium containing 0.72 p.p.m. of nitrogen as ammonium chloride. The maximum rate, about 17 c.mm. of oxygen per hour, was reached between the eighth and tenth hours, then there was a rather marked decline in rate to about 10 c.mm. per hour by the fifteenth hour. From that time on the rate was fairly uniform until the end of the experiment. The rate of increase was less in the medium with 144.0 p.p.m. of nitrogen as ammonium chloride, but continued for a little longer period before the maximum was reached between the fifteenth and eighteenth hour, when oxygen was being consumed at the rate of about 13 c.mm. per hour. From that time on there was a gradual decrease in the rate of consumption until the end of the test. The intermediate amounts of nitrogen employed produced intermediate effects.

Ammonium chloride in all concentrations stimulated oxygen consumption and growth. The smallest amount of ammonium chloride was just as effective as the larger amounts, and even more effective than the largest amount used, 144.0 p.p.m. of nitrogen, which it appears was a larger amount than the organisms could endure for maximum oxygen consumption.

Likewise, oxygen consumption by *Rh. meliloti* was very definitely stimulated by the addition of sodium nitrate to the basic medium. There was an increase in the rate of oxygen consumption during the first 10 or 12 hours, and presumably at that time the organisms were growing. The rate of increase was somewhat proportional to the amount of sodium nitrate in the medium. After the organisms passed the logarithmic period of growth, presumably they passed

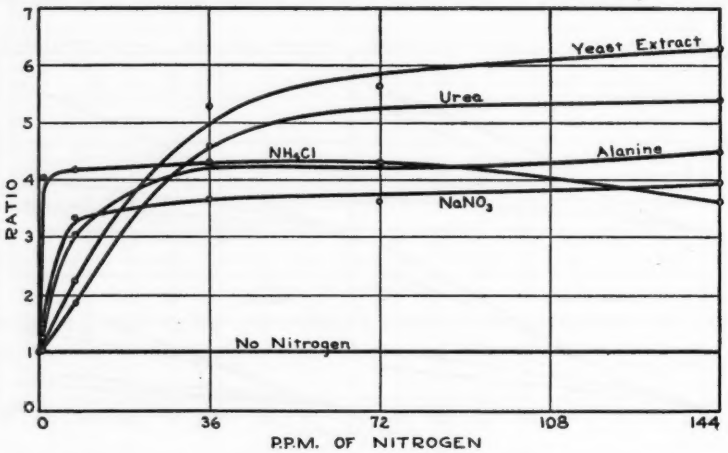

FIG. 4. THE RATIOS OF THE AMOUNTS OF OXYGEN CONSUMED BY *Rhizobium meliloti* IN THE VARIOUS NITROGENOUS MEDIA TO THAT CONSUMED IN THE MEDIUM WITHOUT NITROGEN

into a state of rest and continued to respire at a fairly constant rate for the remainder of the experiment. The total amount of oxygen consumed was considerably larger than when the other species of the genus were grown in the sodium nitrate media.

Similarly, urea and alanine definitely increased the rate of oxygen consumption by the organisms over that in the medium without nitrogen, the maximum rate being proportional to the amount of the nitrogenous materials in the media. This indicates that the nitrogen stimulated growth and that in its presence the organisms were presumably actively multiplying, whereas in the medium without nitrogen they were in a resting state throughout the entire period except, perhaps, for the first hour or two after inoculation, while the nitrogen carried over in the inoculum was being used.

The comparative effects of the different nitrogen sources on oxygen consumption by *Rh. meliloti* are shown in figure 4. The curves in this figure represent the ratio of the amount of oxygen consumed by the organisms in the different nitrogenous media at the end of 22 hours compared with that consumed in the medium without added nitrogen. About the same type of curve is shown for the yeast extract media as was shown for *Rh. trifolii* in figure 1. The curves for the other types of nitrogen are, however, very different. Those in figure 1 indicate that ammonium chloride, sodium nitrate, urea, and alanine had little, if any, stimulation on oxygen consumption and growth of *Rh. trifolii*, whereas the same forms of nitrogen very definitely stimulated oxygen consumption and growth of *Rh. meliloti*.

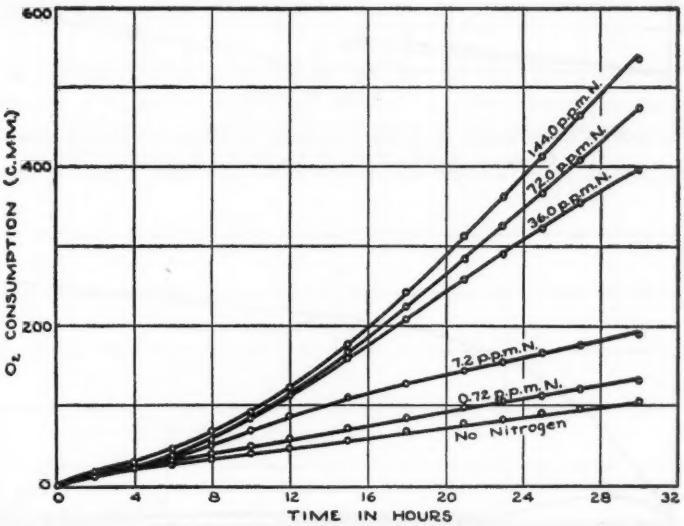

FIG. 5. OXYGEN CONSUMPTION BY *Rhizobium phaseoli* IN MEDIA CONTAINING YEAST EXTRACT

Rhizobium phaseoli.—The results of the oxygen consumption experiments with *Rh. phaseoli* in the various nitrogenous media are shown graphically in figures 5, 6, and 7.

In general, these results are similar to those obtained in the tests with *Rh. trifolii* and *Rh. leguminosarum*. Yeast extract definitely increased the rate of oxygen consumption by the organisms and presumably stimulated growth and multiplication to the same extent. The increase in rate of consumption was directly correlated with the concentration of yeast extract in the medium.

The other nitrogenous materials—ammonium chloride, sodium nitrate, urea, and alanine—had little or no effect on the rate of oxygen consumption. As may be observed, the curves for the nitrogenous materials in figure 6 in prac-

tically every case are very close to the no-nitrogen curve, thus indicating that the cells in the various nitrogenous media were in the same state, which was undoubtedly a resting state, as in the medium without nitrogen. Again, however, there is some evidence that ammonium chloride in the highest concentration used depressed slightly the consumption of oxygen by this organism as it did with *Rh. trifolii* and *Rh. leguminosarum*.

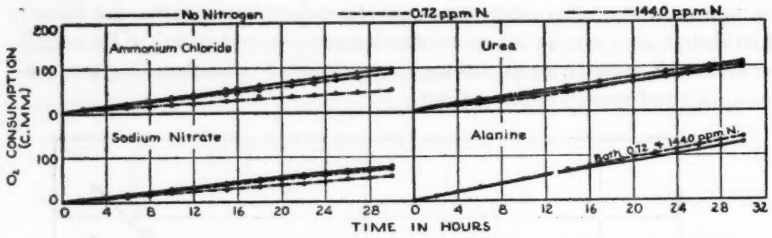

FIG. 6. OXYGEN CONSUMPTION BY *Rhizobium phaseoli* IN MEDIA CONTAINING AMMONIUM CHLORIDE, SODIUM NITRATE, UREA, AND ALANINE

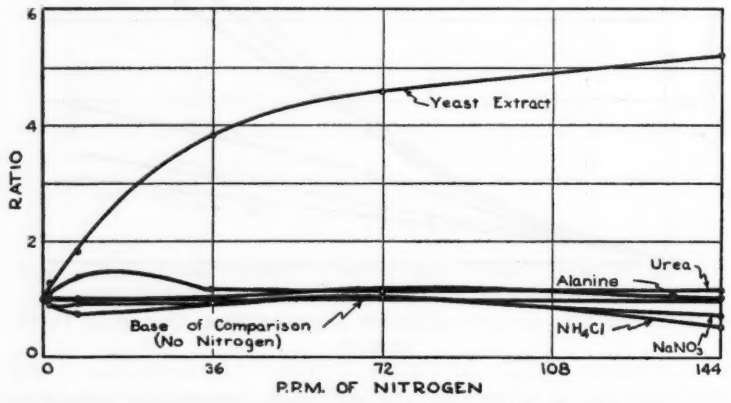

FIG. 7. THE RATIOS OF THE AMOUNTS OF OXYGEN CONSUMED BY *Rhizobium phaseoli* IN THE VARIOUS NITROGENOUS MEDIA TO THAT CONSUMED IN THE MEDIUM WITHOUT NITROGEN

SUMMARY AND CONCLUSIONS

Experiments similar to those with *Rh. leguminosarum* have been conducted with other species of the genus; namely, *Rh. trifolii*, *Rh. meliloti*, and *Rh. phaseoli*, to study the effects of various nitrogen sources on oxygen consumption. The results indicate that, in general, oxygen consumption and therefore growth of *Rh. trifolii* and *Rh. phaseoli* are affected in the same manner as in

the case of *Rh. leguminosarum*. On the other hand, it seems that *Rh. meliloti* reacts entirely differently to the compounds of nitrogen, ammonium chloride, sodium nitrate, urea, and alanine, but in about the same manner to the yeast extract, which is a complex material containing only about 7.6 per cent nitrogen.

The reason for this physiological difference in the organisms of the different species has not yet been explained satisfactorily. Neither is it known whether, after a statistical analysis of physiological reactions of several species, the same general conclusions could be applied to the species as a whole, or whether certain reactions could be definitely linked with the efficiency of certain strains of the organisms in fixing nitrogen symbiotically. These points are being considered further, and it is hoped that additional information concerning the nitrogen nutrition and metabolism of this genus of microörganisms may be obtained.

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ANION EXCHANGE: I. ADSORPTION OF THE PHOSPHORIC ACID IONS BY SOILS

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The adsorption of phosphate by soil has received considerable attention. The fixation by soil has been attributed to the insolubility of the compound formed with iron, aluminum, calcium, and magnesium. More recent investigations have transferred the problem from the field of purely chemical to the field of physico-chemical reactions. Mattson (3, 5) has shown the interrelationship of adsorption of phosphate with the electrokinetic behavior of soil colloids, and the properties of the mutual exchange between various anions (4).

Since cations are exchanged in the soils in nearly equivalent quantities, it would be of considerable interest to establish whether there exists such a law for anion exchange. To attempt to establish such a law, to compare anion exchange with cation exchange, and to study the rôle of the exchangeable cations in the process of anion adsorption are the purposes of this investigation.

SOILS

The experimental work was carried out with four different soils. The two Palestinian soils were obtained from the Kefar-Jehoshua of the Esdraelon Valley, and from the experimental plots of the Agricultural Experiment Station, Rehoboth. The Sharkey and the peat soils were obtained from the New Jersey Agriculture Experiment Station, U. S. A. The mechanical and chemical analyses of the Palestinian soils, together with the base exchange capacity are presented in tables 1 and 2.

All the soils differ widely in their chemical and mechanical composition as well as in their total exchange capacity. The exchange complex of the two Palestinian soils is almost entirely mineral since there is hardly any organic matter in the red sandy soil, and it is scanty in the Esdraelon soil. The exchange complex of the peat is rendered entirely organic, the mineral complex being destroyed by previous treatment with acid. All the soils were converted into H-soils, the Sharkey soil by electrodialysis (2) until all the exchangeable bases were removed, and the Palestinian soils by extraction with a 0.05 *N* solution of hydrochloric acid (1). The peat soil was treated continuously with a 2 per cent solution of boiling hydrochloric acid, and the mineral complex was thus destroyed. After the acid treatment, the soils were washed with distilled water until no free chlorine was present. Ca-soils were prepared from the H-soil by shaking periodically with a solution of $Ca(OH)_2$ for 1 or 2 days. The

soil with the solution was then transferred into dishes and evaporated to dryness. The soil was ground and passed through a 1-mm. sieve.

EXPERIMENTAL

The adsorption experiments were conducted on the air-dry soil, the volume of solution used being always corrected for hygroscopic moisture so that the total volume was 50 cc. The samples were shaken in small bottles for an hour in an automatic shaker with either phosphoric acid or ammonium phosphate, and subsequently allowed to stand for 4 days. This period of contact was

TABLE 1

Mechanical analysis of the soils

SOILS	FINE GRAVEL	COARSE SAND	MEDIUM SAND	FINE SAND	VERY FINE SAND	SILT	CLAY
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Clay soil of Esdraelon Valley (Kefar Jehoshua).....	Passed through a 1-mm. sieve	0.04	0.10	10.15	15.17	19.77	54.77
Red sandy soil of Rehoboth...	0.05	1.15	5.28	66.91	3.46	2.55	20.06

TABLE 2

Chemical analysis of soils and base exchange capacity

SOILS	SiO_2	Al_2O_3	Fe_2O_3	CaO	MgO	P_2O_5	ORGANIC MATTER	CO_2	BASE EXCHANGE CAPACITY M.E. IN 100 GM. SOIL
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	
Clay soil of Esdraelon Valley (Kefar Jehoshua).....	64.98	15.17	8.85	2.17	2.91	0.18	0.86	Traces	51.60
Red sandy soil of Rehoboth.....	88.27	5.51	2.99	0.87	1.08	0.10	0.15	Traces	10.48
Sharkey.....	.....	.....	.....	.....	.....	.....	.....	.....	39.10
Peat.....	.....	.....	.....	.....	.....	.....	.....	.....	132.40

quite sufficient to achieve equilibrium between the soil and phosphoric acid. After 4 days of settling, the supernatant liquid was withdrawn and analyzed, the phosphoric acid being determined gravimetrically, the pH colorimetrically, and calcium as the sulfate in alcoholic solution. All the calculations were made on the basis of oven-dry weight.

Adsorption of phosphoric acid by H-soils

The experiments were carried out with both hydrogen and calcium soils of three different kinds: Esdraelon, Sharkey, and red sandy soil. The H-soils

were shaken with H_3PO_4 or with nearly neutral ammonium phosphate solutions; the Ca-saturated soils were treated only with ammonium phosphate solutions.

The results for the H-sandy soil (series 1) are presented in table 3. As would be expected, the adsorption increases with increasing phosphate concentration, but shows considerable discontinuity, the increase taking place at irregular intervals. Thus in the experiments 3, 4, and 5, where the concentrations of H_3PO_4 vary from 6.00 to 12.00 m.e., the amount of adsorbed PO_4 fluctuates only within the limits of 1.19 to 1.22 m.e. Again at concentrations of 15.00, 17.00, and 20.00 m.e. of phosphoric acid, the quantities adsorped are close to one another; namely, 1.90, 2.01, and 2.00 m.e. respectively. The intermediate

TABLE 3

Adsorption of phosphoric acid ions by a H-sandy soil from solutions of H_3PO_4 of various concentrations

EXPERIMENT NUMBER	INITIAL CONCENTRATION OF PO_4 IN 50 CC.	PO_4 ADSORBED BY 7.5 GM. OF SOIL	ADSORPTION THEORETICALLY CALCULATED*	DIFFERENCE BETWEEN THE PO_4 ACTUALLY ADSOBED AND THAT THEORETICALLY CALCULATED	pH
	m.e.	*m.e.*	*m.e.*	*m.e.*	
1	2.00	0.71	(I) $0.786 \times 1 = 0.786$	−0.08	<2.8
2	4.00	0.82		+0.03	<2.8
3	6.00	1.20		+0.02	<2.8
4	10.00	1.19	(II) $0.786 \times 1.5 = 1.179$	+0.01	<2.8
5	12.00	1.22		+0.04	<2.8
6	13.00	1.63	(III) $0.786 \times 2.0 = 1.572$	+0.06	<2.8
7	15.00	1.91		−0.06	<2.8
8	17.00	2.01	(IV) $0.786 \times 2.5 = 1.965$	+0.04	<2.8
9	20.00	2.00		+0.03	<2.8

* The base exchange capacity of the sandy soil sample is 0.786 me.

amount of adsorption of 1.63 m.e. stands between the aforementioned two groups of figures.

Table 4 presents the results of adsorption experiments with the same soil from nearly neutral ammonia-phosphate solutions.[1] In this series (series 2) the concentration of the phosphoric acid is increased to 0.80 *N*. The general feature of the relationship between adsorption and concentration of solution is similar to that in the experiments of series 1. As a result of relatively high

[1] As a matter of fact, in the experiments with H-soils, when brought in contact with ammonia-phosphate solutions of different pH values, a part or all of the H ions of the complex are replaced by the NH_4 ions. As a result, NH_4(H)-soils are obtained. For convenience, throughout this paper the H-soils were so named, even after their contact with the ammonia-phosphate solutions.

concentrations of ammonia-phosphate, the adsorption becomes negative. Within the limits of the positive adsorption, the amounts of adsorption for concentrations 8.00 and 10.00 m.e. of PO_4 equal 0.78 and 0.76 m.e.; those for concentrations 13.00 and 15.0 m.e. equal 1.52 and 1.58 m.e.; and those for concentrations of 17.00, 20.00, and 23.00 are 1.88, 1.95, and 1.98 m.e., respectively, for a soil sample of 7.5 gm. In the experiments of series 2 with nearly neutral solutions, as in those of series 1 with acid solutions, the same type of adsorption curve is obtained.

TABLE 4

Adsorption of phosphoric acid ions by a H-sandy soil from ammonia phosphate solutions of various concentrations

EXPERIMENT NUMBER	INITIAL CONCENTRATION OF PO_4 IN 50 CC.	PO_4 ADSORBED BY 7.5 GM. OF SOIL	INITIAL CONCENTRATION OF AMMONIA IN 50 CC.	ADSORPTION THEORETICALLY CALCULATED*	DIFFERENCE BETWEEN THE PO_4 ACTUALLY ADSORBED AND THAT THEORETICALLY CALCULATED	pH
	m.e.	*m.e.*	*m.e.*	*m.e.*	*m.e.*	
1	2.00	0.57	1.00	.....	.....	5.6
2	4.00	0.63	2.00	.....	.....	6.0
3	6.00	0.69	3.00	.....	.....	6.5
4	8.00	0.78	4.00	(I) $0.786 \times 1.0 = 0.786$	−0.01	6.6
5	10.00	0.76	5.00		−0.03	6.6
6	12.00	1.19	6.00	(II) $0.786 \times 1.5 = 1.179$	+0.01	6.7
7	13.00	1.52	7.08	(III) $0.786 \times 2.0 = 1.572$	−0.05	7.2
8	15.00	1.58	7.50		+0.01	6.7
9	17.00	1.88	9.27	(IV) $0.786 \times 2.5 = 1.965$	−0.09	6.8
10	20.00	1.95	10.90		−0.02	.....
11	23.00	1.98	12.51		+0.01	.....
12	30.00	0.07	15.00	.....	.....	6.7
13	33.00	−0.30	16.50	.....	.....	6.7
14	36.00	−0.30	18.00	.....	.....	6.7
15	40.00	−0.29	20.00	.....	.....	6.7

*The base exchange capacity of the sandy soil samples is 0.786 m.e.

The phenomenon of more or less constant amounts of adsorption from H_3PO_4 solutions of different concentrations is still more distinctly shown in series 3 (table 5). The H-Esdraelon soil and H-Sharkey soil also give a range of almost constant amounts of adsorption, for a series of solutions of increasing concentrations. Thus, the figures of adsorption for the solutions from 0.16 *N* to 0.46 *N* for the H-Esdraelon soil are close to one another, as is the case also with the figures of adsorption for solutions from 0.12 *N* to 0.20 *N* for the H-Sharkey soil.

Having indicated this tendency of the numbers representing the amounts of adsorption of the PO_4 to fall into groups, we shall examine the given figures in each individual group and the intermediate figure between these groups. It should be mentioned that the total base exchange capacities for the soil samples

TABLE 5

Adsorption of phosphoric acid ions from phosphoric acid solutions of various concentrations by H-clay soils

EXPERIMENT NUMBER	INITIAL CONCENTRATION OF PO_4 IN 50 CC.	PO_4 ADSORBED BY THE SOIL SAMPLE	ADSORPTION THEORETICALLY CALCULATED*	DIFFERENCE BETWEEN THE PO_4 ACTUALLY ADSORBED AND THAT THEORETICALLY CALCULATED	pH
H-Esdraelon soil:	m.e.	m.e.	m.e.	m.e.	
1	1.00	0.68	.....	.....	3.1
2	2.00	1.11	.....	.....	<2.8
3	3.00	1.44	.....	.....	<2.8
4	4.00	1.58	(I) $1.548 \times 1.0 = 1.548$	+0.03	<2.8
5	6.00	1.82	.....	.....	<2.8
6	8.00	2.39		+0.07	<2.8
7	10.00	2.32		±0.00	<2.8
8	13.00	2.36		+0.04	<2.8
9	15.00	2.31	(II) $1.548 \times 1.5 = 2.322$	−0.01	<2.8
10	17.00	2.26		−0.06	<2.8
11	20.00	2.30		−0.02	<2.8
12	23.00	2.33		+0.01	<2.8
13	26.00	2.58	.....	.....	<2.8
H-Sharkey soil:					
1	2.00	1.54	.....	.....	2.9
2	4.00	2.54	.....	.....	<2.8
3	6.00	2.95		+0.02	<2.8
4	8.00	2.91	(I) $2.933 \times 1.0 = 2.933$	−0.02	<2.8
5	10.00	2.94		+0.01	<2.8

* The base exchange capacity of the 3.0-gm. Esdraelon soil samples is 1.548 m.e., and of the 7.5 gm.-Sharkey soil samples, 2.933 m.e.

of the red sandy soil, of the Esdraelon soil, and of the Sharkey soil equal 0.786, 1.548, and 2.933 respectively.

In the acid series of experiments with the H-red sandy soils, the adsorption, expressed in milliequivalents, begins with amounts close to the base exchange

capacity of the soil sample. With an increase in concentration, the adsorption figures increase to about one and a half the total base exchange capacity. With a further increase in concentration, the amount of adsorption of PO_4 is about double the base exchange capacity, and with a still further increase of the concentration the amounts of adsorption equal about two and a half times the exchange capacity of the complex.

In the experiments of series 2, which are carried out with neutral solutions, the amount of adsorption for the identical initial concentrations is less than the exchange capacity of the soil sample. The increase in the concentration of the solution gives an adsorption equal to about the total base exchange capacity; with a further increase, the amount of adsorption is almost equal to one and a half times the total base exchange capacity, in experiment 6; it reaches double the exchange capacity in experiments 7 and 8, and two and a half times the exchange capacity in experiments 9, 10, and 11. The still further increase in the concentration gives a negative adsorption.

In the experiments with the H-Esdraelon soil and H-Sharkey soil the amounts of adsorption increase gradually until they almost equal the base exchange capacity of the soil sample. Then, for the H-Esdraelon soil at solutions of 0.16 *N* to 0.46 *N*, figures are obtained which are equal about to one and a half times the base exchange capacity. A further increase of the concentration leads to an adsorption equal to about double the base exchange capacity (table 7, experiments with Ca-soils). The amounts of adsorption for the H-Sharkey soil from 0.12 to 0.20 *N* solutions about equal the base exchange capacity of the soil sample.

Column 4 in tables 3, 4, and 5 presents the theoretically calculated figures of 1.0, 1.5, 2.0, and 2.5 times the base exchange capacity of the sample. Column 5 gives the difference in milliequivalents between the PO_4 actually adsorbed and that theoretically calculated. The difference between these amounts is insignificant and is within the limits of experimental error.

In order to explain such a regular adsorption, which increases by halves of the total base exchange capacity for each consecutive group, we must take into account the fact that phosphoric acid does not dissociate at once into an anion of a higher valence and into a corresponding amount of cations, but dissociates into H_2PO_4, HPO_4 and PO_4 ions. The phosphoric acid, therefore, may be adsorbed from a solution either as a monovalent, divalent, or trivalent ion, or as a combination of all three. The adsorption of the phosphoric acid ions of the different valences depends upon various factors like the pH, the concentration of the solution, and the nature of the soil material.

For the first group of constant quantities of adsorption the amount of adsorbed PO_4 equals about the base exchange capacity of the soil sample. This is possible when the exchange between the phosphoric acid ions and the hydroxyl ions of the soil complex, which are in an equilibrium with the exchangeable bases, proceeds in equivalent amounts. In this case the phosphoric acid is adsorbed as trivalent ions. For the second group the amount of adsorbed

PO_4 equals about one and a half times the base exchange capacity of the soil. This would be the case if all the anions of the soil complex were replaced by the divalent phosphoric acid ions. The quantities of adsorption for the third and fourth groups are twice and two and a half times the exchange capacity of the soil samples respectively. In these cases the adsorption consists probably of a combination of phosphoric acid ions of the varying valences.

The established rule of the equivalent relationship between the adsorption of PO_4 and the base exchange capacity of the soil complex indicates that the cations and the anions, which are in a state of electrostatic equilibrium, are present in equivalent quantities. The equivalent exchange reaction, which is characteristic of the cations (1, 2), exists also for the anions.

The difference in character of adsorption among the various groups indicates the existence of definite forms of PO_4 adsorption. For the H-soils the four distinct groups represent four definite forms of adsorption.

The weak concentrations of the solution give smaller quantities of adsorption than the exchange capacity of the soil sample. Here too, as in the case of the complete forms of adsorption, the process of adsorption proceeds on the same principles of an equivalent anion exchange. However, because of the weak concentration of the phosphoric acid solution, the exchange between the anions of the soil complex and the phosphoric acid ions of the solution is incomplete. As the concentration of the phosphoric acid ions increases the anion exchange becomes complete.

Adsorption of phosphoric acid by Ca-soils

Series 4 and 5 (tables 6 and 7) present the adsorption of PO_4 from neutral ammonia phosphate solutions by sandy and clay Ca-soils. The soils are similar to those used in series 1, 2, and 3 where they appeared as unsaturated. The course of adsorption of PO_4 by the Ca-soils is in general similar to that of the H-soils. For the given series of Ca-soils, the curves representing the characteristic phenomenon of the distribution of the amounts of adsorption into groups are more distinct than in the H-soils (fig. 1). The quantities of adsorption of these groups are in definite ratios with the exchange capacity of the soil complex. These ratios for the five various forms of adsorption equal about 1.5, 2.0, 2.5, 3.0, and 3.5 respectively. These figures are much higher than those obtained for the H-soils with many identical concentrations of neutral or even acid solutions of phosphate. The character of adsorption for the Ca-soils is somewhat different from that for the unsaturated soils and has become complicated by the presence of Ca in the complex. The rôle which the exchangeable calcium plays in the given experiments is obvious. Its introduction into the complex increased the adsorption of the PO_4.

In some cases the amount of PO_4 adsorbed by H-soils equals that adsorbed by Ca-soils from solutions of identical concentrations. This is due to the fact that the Ca-soil and H-soil give, at similar concentrations, different forms of adsorption which yield equal amounts of adsorbed PO_4 (*see* tables 3, 5, 6, and 7

and fig. 1). This phenomenon depends upon three factors which affect the adsorption: (*a*) the pH of the solution; (*b*) the valence of the adsorbed phosphoric acid ion; and. as will be explained later, (*c*) the combination of the replaced exchangeable calcium with the phosphoric acid.

TABLE 6

Adsorption of phosphoric acid ions by a Ca-sandy soil from ammonia-phosphate solutions of various concentrations*

EXPERIMENT NUMBER	INITIAL CONCENTRATION OF PO_4 IN 50 CC.	PO_4 ADSORBED BY 7.5 GM. OF SOIL	RATIO OF EXCHANGE Ca TO ADSORBED PO_4	ADSORPTION THEORETICALLY CALCULATED†	DIFFERENCE BETWEEN THE PO_4 ACTUALLY ADSORBED AND THAT THEORETICALLY CALCULATED	pH
	m.e.	*m.e.*	*m.e.*	*m.e.*	*m.e.*	
1	1.50	0.42	.....	.....	.....	7.05
2	2.00	0.52	.....	.....	.....	7.05
3	3.00	0.95	.....	.....	.....	7.05
4	4.00	1.06	.....	.....	.....	7.05
5	5.00	1.20	0.66	(I) $0.786 \times 1.5 = 1.179$	+0.02	7.05
6	6.00	1.17	0.67		−0.01	7.05
7	7.00	1.24	0.63		+0.06	7.05
8	8.00	1.51	.....	(II) $0.786 \times 2.0 = 1.572$	−0.06	7.05
9	9.00	1.54	.....		−0.03	7.05
10	10.00	1.49	.....		−0.08	7.05
11	11.00	1.57	.....		±0.00	7.05
12	13.00	1.58	.....		+0.01	7.05
13	15.00	1.90	.....	(III) $0.786 \times 2.5 = 1.965$	−0.07	7.0
14	17.00	1.98	.....		+0.01	7.0
15	20.00	2.32	.....	(IV) $0.786 \times 3.0 = 2.358$	−0.04	.....
16	23.00	2.41	.....		+0.05	.....
17	26.00	2.72	.....	(V) $0.786 \times 3.5 = 2.751$	−0.03	.....
18	30.00	2.73	.....		−0.02	7.05
19	33.00	0.39	.....	.....	.....	.....
20	36.00	0.38	.....	.....	.....	6.9
21	40.00	0.44	.....	.....	.....	6.9

* Ratio of PO_4 to NH_4 equals 1.0:0.5 m.e.

† The exchange capacity of the soil samples is 0.786 m.e.

In order to establish the rôle of the exchangeable calcium in the adsorption of the PO_4, the experiments (series 6) presented in table 8 were undertaken. For these experiments a concentration of the solution was chosen which yields an adsorption belonging to the first group of constant quantities for Ca-soils. In this series, samples of Ca-sandy soil were shaken with various amounts of

$Ca(OH)_2$ solution of a definite concentration. The calcium hydroxide was quickly adsorbed by the Ca-soil samples. In the solution only traces of calcium were left, just as in experiment 1 where the Ca-soil was shaken with distilled water only (table 9). Complete adsorption of calcium took place only at a definite concentration of $Ca(OH)_2$. The highest concentration of the $Ca(OH)_2$ solution, from which the calcium was completely adsorbed by a Ca-

TABLE 7

Adsorption of phosphoric acid ions by a Ca-clay (Esdraelon) soil from ammonia phosphate solutions of various concentrations*

EXPERIMENT NUMBER	INITIAL CONCENTRATION OF PO_4 IN 50 CC.	PO_4 ADSORBED BY 3.0 GM. OF SOIL	RATIO OF EXCHANGE Ca TO ADSORBED PO_4	ADSORPTION THEORETICALLY CALCULATED†	DIFFERENCE BETWEEN THE PO_4 ACTUALLY ADSORBED AND THAT THEORETICALLY CALCULATED	pH
	m.e.	*m.e.*	*m.e.*	*m.e.*	*m.e.*	
1	2.00	0.79	.....	.....	.....	7.0
2	4.00	1.57	.....	.....	.....	7.0
3	6.00	2.09	.....	.....	.....	7.0
4	7.00	2.31	0.67		−0.01	7.0
5	8.00	2.31	0.67		−0.01	7.0
6	9.00	2.32	0.67	(I) $1.548 \times 1.5 = 2.322$	±0.00	7.05
7	10.00	2.37	0.65		+0.05	7.05
8	11.00	2.33	0.66		+0.01	.....
9	13.00	2.90	.....	.....	.....	.....
10	15.00	3.01			−0.09	6.9
11	20.00	3.02		(II) $1.548 \times 2.0 = 3.096$	−0.08	6.9
12	23.00	3.76			−0.11	6.9
13	26.00	3.90		(III) $1.548 \times 2.5 = 3.870$	+0.03	6.9
14	30.00	3.91			+0.04	6.9
15	33.00	4.70			+0.06	6.9
16	36.00	4.58		(IV) $1.548 \times 3.0 = 4.644$	−0.06	6.9
17	40.00	4.64			±0.00	6.9

* Ratio of PO_4 to NH_4 in the solutions equals 1.0:0.5 m.e.

† The exchange capacity of the soil samples is 1.548 m.e.

soil sample of 7.5 gm. equals 0.323 m.e. of Ca in 40 cc. of solution. The total amount of adsorbed calcium by the soil complex for the last experiment was brought to 1.109 m.e., which corresponds to 141.1 per cent of the total base exchange capacity for the soil sample, established at pH 7.0. The adsorbed calcium within the limits of the exchange capacity of the soil complex, and that which is above its limits, proved to be identical in relation to its exchange

properties. As a result of contact with neutral salt solutions it totally passed into the solution.

The Ca-soils in which the exchangeable calcium, in milliequivalents, exceeds the total base exchange capacity of the soil, established at pH 7.0, are designated as "over-limed" Ca-soils.

After all the prepared soil samples had been shaken with ammonium phosphate solutions, the amount of adsorbed PO_4 was measured. As the amount of adsorbed calcium increased, the adsorption of PO_4 increased. The ratio of

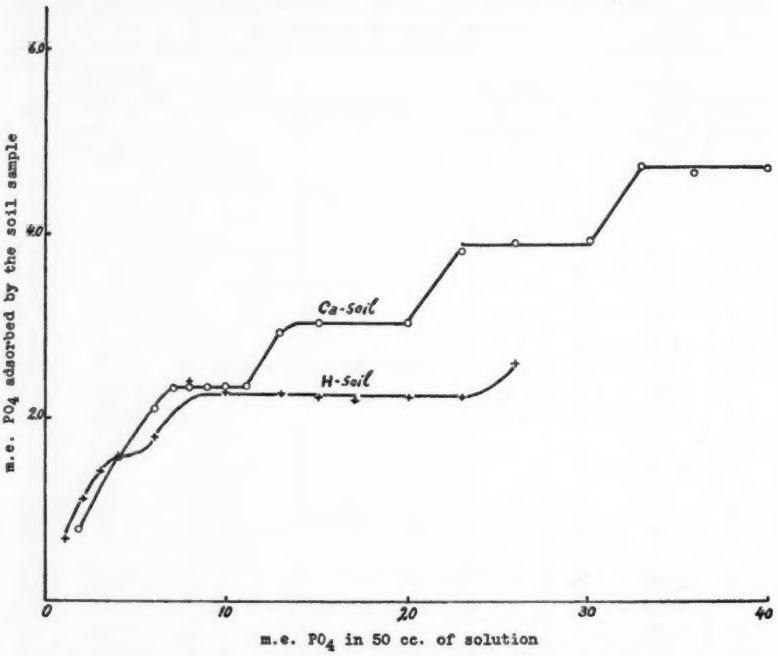

FIG. 1. ADSORPTION OF PHOSPHORIC ACID IONS BY A H-SOIL AND A CA-SOIL (FROM ESDRAELON VALLEY) FROM SOLUTIONS OF VARIOUS CONCENTRATIONS

the total amount of the exchangeable calcium to the amount of adsorbed PO_4 was found to remain the same as for the experiments with Ca-soils of the series 4 and 5 with concentrations yielding the first form of adsorption. This ratio is equal, or very close, to 0.67, which means that every 2 m.e. of adsorbed calcium tied 3 m.e. of phosphoric acid. The exact ratio in this case should be 0.666, but for practical purposes 0.67 is used. This constant ratio indicates the dependence of the adsorbed PO_4 upon the amount of exchangeable calcium.

Column 5, tables 6 and 7, and column 7, table 8, present the theoretically calculated amounts of PO_4 which should be adsorbed by a sample of either

Ca-soil or "over-limed" Ca-soil, when the adsorption depends only upon the amount of exchangeable calcium, i.e., when the PO_4 combines with the Ca in proportions of 3:2 m.e. The differences between the amounts of adsorption

TABLE 8

The adsorption of phosphoric acid ions by "over-limed" Ca-sandy soils

EXPERIMENT NUMBER	DEGREE OF SATURATION OF THE SOIL COMPLEX WITH CALCIUM		INITIAL CONCENTRATION OF PO_4 IN 50 CC.	PO_4 ADSORBED BY 7.5 GM. OF SOIL	RATIO OF EXCHANGE Ca TO ADSORBED PO_4	ADSORPTION THEORETICALLY CALCULATED ACCORDING TO THE RATIO OF $\frac{\text{m.e. exch. Ca}}{\text{m.e. ads. } PO_4} = 0.67$	DIFFERENCE BETWEEN THE PO_4 ACTUALLY ADSORBED AND THAT THEORETICALLY CALCULATED	pH
	Per cent of base exchange capacity of the soil	M.e. of exchange Ca in the soil sample						
			m.e.	*m.e.*			*m.e.*	
1	100.0	0.786	6.00	1.17	0.67	1.18	−0.01	7.05
2	102.8	0.807	6.00	1.26	0.64	1.21	+0.05	7.05
3	106.7	0.839	6.00	1.30	0.65	1.26	+0.04	7.05
4	113.5	0.892	6.00	1.34	0.67	1.34	±0.00	
5	127.1	0.999	6.00	1.59	0.64	1.50	+0.09	
6	141.1	1.109	6.00	1.63	0.68	1.66	−0.03	

TABLE 9

Adsorption of Ca by a Ca-sandy soil from a $Ca(OH)_2$ solution

EXPERIMENT NUMBER	INITIAL CONCENTRATION OF $Ca(OH)_2$ IN 40 CC. OF SOLUTION	FINAL CONCENTRATION OF Ca IN 40 CC. OF SOLUTION	pH
	m.e.	*m.e.*	
1	.....	Trace	7.15
2	0.021	Trace	7.15
3	0.053	Trace	7.15
4	0.107	Trace	7.15
5	0.213	Trace	7.15
6	0.323	Trace	7.15

obtained by these experiments and the calculated amounts are insignificant. The regularity in the adsorption, which is expressed by the ratio

$$\frac{\text{exchange Ca}}{\text{adsorbed } PO_4} = 0.67$$

for the Ca-soils as well as for the "over-limed" Ca-soils, indicates that in both cases the nature of the combination of the exchangeable calcium with the adsorbed PO_4 is identical.

The character of the next forms of adsorption for the Ca-soils is altogether different (series 4 and 5). The ratio of adsorbed PO_4 to the base exchange capacity of the soil increases with each consecutive form of adsorption by about one half. The nature of adsorption for the consecutive forms and the

rôle of the exchangeable calcium in PO_4 fixation will be elucidated when the mechanism of the PO_4 adsorption for the different forms is discussed. The first and most important step in explaining this mechanism is to establish the fate of the exchangeable calcium when it comes in contact with the ammonium phosphate solutions. The question is whether there is a simultaneous combination of the exchangeable calcium with the exchange complex and the adsorbed phosphoric acid ions, or whether the Ca ions combine with the phosphoric acid only and thus break apart from the complex. Under neutral conditions, which is the case in the present experiments, it is impossible to solve analytically by a direct method the problem of calcium replacement in the presence of PO_4 in the solution. However, an indirect answer to the problem presented may be obtained from the results of the experiments presented in tables 10, 11, 12 and 13. These experiments were undertaken to investigate the influence of the reaction and the degree of unsaturation of the complex on the PO_4 adsorption.

TABLE 10

Adsorption of phosphoric acid ions by a H-Sharkey soil from ammonium phosphate solutions of various pH values

EXPERIMENT NUMBER	INITIAL CONCENTRATION OF PO_4 IN 50 CC.	INITIAL CONCENTRATION OF AMMONIA IN 50 CC.	PO_4 ADSORBED BY 7.5 GM. OF SOIL*	pH
	m.e.	*m.e.*	*m.e.*	
1	6.00	....	2.95	<2.8
2	6.00	2.00	2.88	3.3
3	6.00	3.00	2.76	3.9
4	6.00	4.00	2.47	5.5
5	6.00	5.00	2.46	5.8
6	6.00	6.00	2.20	>7.0
7	6.00	7.00	1.70	>7.0
8	6.00	7.88	1.33	>8.6

* The exchange capacity of the soil sample is 2.933 m.e.

Influence of pH and degree of unsaturation of the complex on PO_4 adsorption

Table 10 (series 7) shows the adsorption of PO_4 by the H-Sharkey soil from ammonium phosphate solutions of various pH values. The pH was changed by introducing different amounts of ammonia into the solution. The variation of adsorption with changes in pH indicates the influence of the OH concentration on the adsorption of PO_4 by H-soils.

Series 8 (table 11) shows the adsorption by soils in which the exchangeable hydrogen is partially or completely replaced by calcium. The samples were shaken with slightly or strongly alkaline ammonium phosphate solutions. A difference in adsorption as influenced by the pH is noted, especially in the completely unsaturated samples and in those in which only 25 per cent and 50 per cent of the hydrogen ions were replaced by calcium. For the samples in which

75 per cent of the exchangeable hydrogen ions were replaced by Ca, only a very slight difference is noted in the adsorption of PO_4. No difference in the adsorption was obtained for the completely saturated samples. In the partly unsaturated soils, the ratio of exchangeable Ca to adsorbed PO_4 equals, or is close to, 0.67. The adsorption for these soils depends only on the amount of the exchangeable calcium, as was found in the case of the Ca-soils and "overlimed" Ca-soils.

As the amount of of the exchangeable calcium in the complex decreases, the ratio $\frac{\text{exchange Ca}}{\text{adsorbed } PO_4}$ becomes smaller than 0.67. If we assume that every 2 m.e. of exchangeable calcium combines with 3 m.e. of PO_4, as established for the Ca-soils at the same concentration of PO_4 in the solution (tables 6, 8), the

TABLE 11

Different degrees of saturation with Ca of the sandy soil and phosphoric acid ion adsorption from ammonium phosphate solutions of various pH values

EXPERIMENT NUMBER	DEGREE OF SATURATION OF THE SOIL COMPLEX WITH CALCIUM		RATIO OF PO_4 TO NH_4	PO_4 ADSORBED BY 7.5 GM. OF SOIL	RATIO OF EXCHANGEABLE Ca TO ADSORBED PO_4	pH
	Per cent of base exchange capacity of the soil	m.e. of Ca in the soil sample				
			m.e.	*m.e.*		
1	0.0	0.000	6.00:4.00	0.29	....	7.3
2	25.0	0.197	6.00:4.00	0.64	0.31	7.4
3	50.0	0.393	6.00:4.00	0.76	0.51	7.4
4	75.0	0.590	6.00:4.00	0.85	0.69	7.5
5	100.0	0.786	6.00:4.00	1.15	0.68	7.7
6	0.0	0.000	6.00:6.00	0.12	....	>8.6
7	25.0	0.197	6.00:6.00	0.53	0.37	>8.6
8	50.0	0.393	6.00:6.00	0.66	0.59	>8.6
9	75.0	0.590	6.00:6.00	0.88	0.67	>8.6
10	100.0	0.786	6.00:6.00	1.15	0.68	>8.6

amount of PO_4 combined with calcium can be calculated. By subtracting this calculated amount from the total amount of PO_4 adsorbed by the soil sample (table 11, experiments 2, 3, 7, 8), an excess of PO_4 will be obtained. This excess belongs to the PO_4 which is in combination with the part of the complex free from exchangeable calcium.

The adsorption of PO_4 by soils completely saturated with Ca is presented in series 9 (table 12). The pH of the solutions in this series of experiments fluctuates within wide limits. Changes in the pH within the limits of >8.6 to 6.4 did not influence the amount of adsorbed PO_4. The characteristic ratio of $\frac{\text{exchangeable Ca}}{\text{adsorbed } PO_4} = 0.67$, which was obtained for all pH values up to 6.1 inclusive, becomes smaller as the solution becomes more acid. At pH 6.1 re-

placed calcium appeared in the solution, and the amount increased with the decrease of pH. With the loss of a part of the exchangeable calcium the adsorption becomes more complicated. One part of the phosphoric acid combines with the Ca remaining in the complex, and the other one with the unsaturated part of the soil complex.

TABLE 12

Adsorption of phosphoric acid ions by a Ca-sandy soil from ammonium phosphate solutions of various pH values

EXPERIMENT NUMBER	INITIAL CONCENTRATION OF THE PO_4 IN 50 CC.	INITIAL CONCENTRATION OF AMMONIA IN 50 CC.	PO_4 ADSORBED BY 7.5 GM. OF SOIL	RATIO OF EXCHANGE Ca TO ADSORBED PO_4	AMOUNT OF REPLACED Ca IN 50 CC. OF SOLUTION	AMOUNT OF EXCHANGE Ca LEFT IN THE SOIL COMPLEX	ADSORPTION THEORETICALY CALCULATED ACCORDING TO THE RATIO OF $\frac{\text{m.e. exch. Ca}}{\text{m.e. ads. } PO_4} = 0.67$	DIFFERENCE BETWEEN THE PO_4 ACTUALLY ADSORBED AND THAT THEORETICALLY CALCULATED	pH
	m.e.	*m.e.*	*m.e.*		*m.e.*	*m.e.*		*m.e.*	
1	6.00	6.00	1.15	0.68	.....	0.786	1.179	−0.03	>8.6
2	6.00	5.00	1.15	0.68	.....	0.786	1.179	−0.03	>8.6
3	6.00	4.00	1.15	0.68	.....	0.786	1.179	−0.03	7.7
4	6.00	3.00	1.17	0.67	.....	0.786	1.179	−0.01	7.05
5	6.00	2.80	1.20	0.65	.....	0.786	1.179	+0.02	6.7
6	6.00	2.60	1.15	0.68	.....	0.786	1.179	−0.03	6.4
7	6.00	2.40	0.91	0.67	0.174	0.612	0.922	−0.01	6.1
8	6.00	2.20	0.75	0.62	0.325	0.461	0.691	+0.06	5.9
9	6.00	2.00	0.72	0.60	0.349	0.437	0.655	+0.06	5.4
10	6.00	1.80	0.72	0.54	0.395	0.391	0.587	+0.13	4.8
11	6.00	1.60	0.85	0.46	0.395	0.391	0.587	+0.26	4.1

TABLE 13

Adsorption of phosphoric acid ions, by a H-Sharkey soil partly saturated with calcium, from ammonia phosphate solutions of various pH values

EXPERIMENT NUMBER	DEGREE OF SATURATION OF THE SOIL COMPLEX WITH CALCIUM	INITIAL CONCENTRATION OF PO_4 IN 50 CC.	INITIAL CONCENTRATION OF AMMONIA IN 50 CC.	PO_4 ADSORBED BY 7.5 GM. OF SOIL	pH
	per cent	*m.e.*	*m.e.*	*m.e.*	
1	75.0	6.00	5.00	2.91	7.4
2	75.0	6.00	6.00	2.61	7.6
3	75.00	6.00	7.00	2.48	>8.6
4	75.00	6.00	7.88	2.32	>8.6

The decrease of the absolute amount of adsorbed PO_4 as the pH decreases below 6.4 is explained by the gradual loss of the exchangeable Ca. While 2 m.e. of exchangeable calcium in Ca-soils fix 3 m.e. of PO_4, in H-soils, under similar conditions, 1 m.e. of the exchangeable anions of the complex is replaced by 1 m.e. of the PO_4 ions.

The ratio of $\frac{\text{exchangeable Ca}}{\text{adsorbed } PO_4} = 0.67$ is still retained for sandy soil samples having a Ca saturation in the limits of 75 per cent of their base exchange capacity. The limits of Ca saturation which produce such a ratio are entirely different for the Sharkey soil, the exchange capacity and composition of which differ from those of the red sandy soil. Electrodialyzed soil samples, after having been saturated with calcium to 75 per cent of their exchange capacity, were shaken with ammonium phosphate solutions of different pH values (table 13). The concentration of PO_4 in the solution is the same as in the previous series of experiments (tables 11, 12). At this concentration the PO_4 combines with the exchangeable calcium only in the ratio $\frac{Ca}{PO_4} = \frac{2}{3}$. However, at the same time an additional adsorption with the unsaturated part of the complex is observed. This can be judged from the changes in amount of adsorption with the changes in pH. Various forms of adsorption are thus obtained for the two different soils saturated to an equal degree by calcium. The lack of similarity in the forms of adsorption for these soils is directly related to the differences in the exchange capacity and the amount of the hydrogen ions present in the complex.

Mechanism of PO_4 adsorption by Ca-soils

From the experiments with Ca-soils, with "over-limed" Ca-soils, and with soils partially unsaturated with calcium (tables 6, 7, 8, 10, and 11), it is apparent that at the first form of adsorption the exchangeable calcium appears as the sole factor which regulates the amount of PO_4 adsorption. The adsorption regulated only by the exchangeable calcium is possible when the calcium does not split off from the soil complex when it comes into contact with the ammonium phosphate solutions. The union of the exchangeable calcium by its two valences with the phosphoric acid ions in the ratio of 2:3 would result in splitting off from the complex the newly formed salt which is practically insoluble, leaving behind a soil complex completely or partly unsaturated with calcium. The soil complex free from calcium would give, in turn, a new additional adsorption characteristic of H-soils. Such an additional adsorption however, is not observed. The connection between the exchangeable calcium and the complex is confirmed by the experiments on the influence of the pH on the adsorption by Ca-soils and H-soils.

The exchangeable calcium left in the complex combines simultaneously also with the adsorbed PO_4. Such a twofold connection is possible when one valence of the calcium ion remains in electrostatic equilibrium with one of its two hydroxyls, and the other valence with the phosphoric acid ion which had replaced its other hydroxyl. The phosphoric acid ion which replaces one of the two hydroxyls of the divalent calcium, giving with it the established ratio $\frac{\text{exchange Ca}}{\text{adsorbed } PO_4} = 0.67$, is the monovalent H_2PO_4 ion.

Table 14 explains the fate of the exchangeable calcium when it comes in contact with ammonium phosphate solutions yielding the second form of adsorption for Ca-soils. As the pH increases, the adsorption of PO_4 decreases. Such an adsorption is typical for H-soils and is possible for the Ca-soils only when the exchangeable calcium is split off from the complex; this occurs when it comes in contact with solutions of higher concentrations.

The process of complete replacement of the exchangeable calcium from the complex in the transitional stage between the first and second forms of adsorption, as seen in tables 6 and 7, occurs within narrow limits of concentrations for the red sandy soil, and within slightly wider limits for the Esdraelon soil.

TABLE 14

Forms of PO_4 adsorption at various H-ion concentrations

EXPERIMENT NUMBER	INITIAL CONCENTRATION OF PO_4 IN 50 CC.	INITIAL CONCENTRATION OF AMMONIA IN 50 CC.	PO_4 ADSORBED BY THE SOIL SAMPLE	ADSORPTION THEORETICALLY CALCULATED*	DIFFERENCE BETWEEN THE PO_4 ACTUALLY ADSORBED AND THAT THEORETICALLY CALCULATED	pH
	m.e.	*m.e.*	*m.e.*	*m.e.*	*m.e.*	
Ca-sandy soil						
1	9.00	4.50	1.54	(II) $0.786 \times 2.0 = 1.572$	−0.03	7.08
2	9.00	6.00	0.94		−0.63	7.7
3	9.00	9.00	0.76		−0.81	>8.6
Ca-clay Esdr. soil						
1	15.00	7.50	3.01	(II) $1.548 \times 2.0 = 3.096$	−0.09	6.9
2	15.00	10.00	2.66		−0.44	
3	15.00	15.00	2.47		−0.63	>8.6

* The exchange capacity is 0.786 m.e. for the sandy soil samples, and 1.548 m.e. for the Esdraelon soil samples.

As the exchangeable calcium is being replaced from the complex under neutral conditions of the ammonium phosphate solutions, such as in the present experiments, it forms an insoluble tribasic salt of calcium phosphate. This salt contains PO_4 in quantities equal, in milliequivalents, to the exchange capacity of the soil complex. By subtracting from the total adsorbed PO_4 the amount which is bound to the replaced calcium, the amount of PO_4 adsorbed by the H-complex will be obtained. This amount for the second, third, fourth, and fifth forms of adsorption will equal about 1.0, 1.5, 2.0, and 2.5 times the exchange capacity of the soil complex, respectively. An identical order of adsorption, as influenced by the exchange capacity and the valence of the adsorbed anion, is established for the H-soils.

Negative adsorption of the phosphoric acid ion

The last regular form of adsorption for the red sandy soils occurs when the PO_4 combines with the complex in an amount which is equal to about 2.5 times the exchange capacity of the complex for the H-soils, and 3.5 times for the Ca-soils. As the concentration of the solution increases the adsorption becomes negative (tables 4 and 6). A definite positive amount of adsorbed PO_4 for the Ca-soil in the given case should be accounted for by its combination with the replaced calcium. The difference between the amount of PO_4 actually adsorbed and that which the replaced calcium should fix in its transition to tricalcium phosphate will give, for the Ca-soil samples, their negative adsorption values, which are close to those obtained for the H-soils at the same concentration of the solution. The phenomenon of negative adsorption depends not only on the definite concentration of the solution but also on the exchange capacity of the soil sample. That concentration of ammonium phosphate solution which gives a negative adsorption for 7.5-gm. samples of a sandy soil with an exchange capacity of 0.786 m.e. yields only the third form of adsorption for a 3-gm. sample of the Ca-clay soil with an exchange capacity of 1.548 m.e.

Speed of anion exchange reaction

Two series of experiments presented in table 15, were undertaken to determine the time necessary to establish an equilibrium between the phosphoric acid ions of an acid or neutral solution and the exchangeable hydroxyls of the exchange complex; in other words, to determine the speed of the anion exchange reaction. The experiments were made on Ca-soil and H-soil samples. The Ca-soils were brought in contact with a neutral ammonium phosphate solution, and the H-soils with a solution of H_3PO_4. According to their concentrations, the solutions yield the first form of adsorption for the Ca-soils as well as for the H-soils. In experiments 1 to 4 of both series, the soil samples were shaken with the solutions for 2, 5, 15, and 60 minutes, respectively. After this the solutions were immediately filtered to determine the amount of adsorbed PO_4. In experiments 5 to 7, the soil samples were shaken with the solutions for 1 hour but afterwards were left in contact with the solutions for 2, 4 and 6 days. After this time the upper clear solution was decanted and the amount of PO_4 determined. In spite of the fact that the solutions in both series of experiments are, according to their concentration of PO_4, very close to each other, the rapidity of anion exchange for the calcium saturated and unsaturated soils differs greatly. For the Ca-soils, the speed of exchange is very great. About 90 per cent of the hydroxyl ions which take part in the exchange are replaced by the H_2PO_4 ions in the first 2 minutes of contact between the soil and solution. However, a difference was observed for the H-soils where three hydroxyl ions of the complex are replaced by one trivalent PO_4 ion. In this case the replacement proceeded much more slowly, and a complete replacement of the exchangeable hydroxyl ions occurred only after 4 days of contact between the soil with the solution.

TABLE 15

The speed of anion exchange reaction

EXPERIMENT NUMBER	INITIAL CONCENTRATION OF PO_4 IN 50 CC.	INITIAL CONCENTRATION OF AMMONIA IN 50 CC.	TIME OF CONTACT BETWEEN THE SOIL AND THE PO_4 SOLUTION	PO_4 ADSORBED BY THE SOIL SAMPLE	THEORETICALLY CALCULATED ADSORPTION ACCORDING TO THE FIRST FORM*	DIFFERENCE BETWEEN THE PO_4 ACTUALLY ADSORBED AND THAT THEORETICALLY CALCULATED	PO_4 ADSORBED IN RELATION TO PO_4 THEORETICALLY CALCULATED	pH
	m.e.	*m.e.*		*m.e.*	*m.e.*		*per cent*	
Ca-sandy soil								
1	6.00	3.00	2 min.	1.04		−0.14	88.2	.....
2	6.00	3.00	5 min.	1.07		−0.11	90.8	.....
3	6.00	3.00	15 min.	1.07		−0.11	90.8	.....
4	6.00	3.00	60 min.	1.12	0.786 × 1.5 = 1.179	−0.06	95.0	.....
5	6.00	3.00	48 hours	1.20		+0.02	101.8	.....
6	6.00	3.00	96 hours	1.17		−0.01	99.2	7.05
H-sandy soil								
1	4.00	....	2 min.	0.13		−0.66	16.5	<2.8
2	4.00	....	5 min.	0.25		−0.54	31.8	<2.8
3	4.00	....	15 min.	0.31		−0.48	39.4	<2.8
4	4.00	....	60 min.	0.31	0.786 × 1.0 = 0.786	−0.48	39.4	<2.8
5	4.00	....	48 hours	0.61		−0.18	77.6	<2.8
6	4.00	....	96 hours	0.80		+0.01	101.4	<2.8
7	4.00	....	144 hours	0.79		±0.00	100.0	<2.8

* The exchange capacity of the soil samples is 0.786 m.e.

TABLE 16

Adsorption of phosphoric acid ions, by a H-peat and a Ca-peat, from ammonium phosphate solutions of various pH values

EXPERIMENT NUMBER	INITIAL CONCENTRATION OF PO_4 IN 50 CC.	INITIAL CONCENTRATION OF AMMONIA IN 50 CC.	FINAL CONCENTRATION OF PO_4 IN 50 CC.	PO_4 ADSORBED BY 1.5 GM. OF PEAT*	pH
	m.e.	*m.e.*	*m.e.*	*m.e.*	
H-peat					
1	6.00	....	5.91	+0.09	<2.8
2	6.00	2.00	5.86	+0.14	.....
3	6.00	4.00	6.10	−0.10	<7.0
4	6.00	6.00	6.12	−0.12	>7.0
5	6.00	7.69	6.29	−0.29	>7.0
Ca-peat					
1	6.00	....	5.86	+0.14	3.0
2	6.00	2.00	5.89	+0.11	.....
3	6.00	4.00	6.09	−0.09	7.0
4	6.00	6.00	6.10	−0.10	>7.0
5	6.00	7.69	6.28	−0.28	>7.0

* The base exchange capacity of the peat samples is 1.986 m.e.

PO_4 adsorption by hydrogen and calcium organic exchange complexes

The organic exchange complex, in contrast to the mineral, possesses practically no capacity to adsorb PO_4 (table 16). A certain insignificant positive adsorption under acid conditions changes into a negative absorption as the neutral point approaches. In alkaline solutions the negative adsorption increases. The introduction of calcium into the organic exchange complex, a factor which increases the amount of PO_4 adsorption in the case of the mineral complex, did not change the adsorption in the present case. The degree of dissociation of the anions and cations of the exchange organic complex is probably very insignificant, and the connection of these ions with the organic complex is much more stable than with the mineral soil complex. This stability of connection of the exchangeable cations with the organic complex was observed in the experiments with acid solutions; the calcium remained entirely in the organic complex, whereas in the experiments with the mineral Ca-complex, at similar concentrations of PO_4 in the solution and at similar pH values, considerable amounts of exchangeable calcium passed into the solution as a result exchange with the H ions.

SUMMARY

The rôle of the mineral and organic exchangeable complexes and their exchangeable H and Ca cations in the adsorption of phosphoric acid ions is discussed. A detailed study is presented of the relationship between the amount of adsorbed PO_4 and its concentration in the solution, as well as of the dependence of this adsorption on the exchange capacity of the soil complex, on the amount of the exchangeable Ca and H, and on the pH of the solution.

The experiments were carried out on one peat and three mineral soils. Definite amounts of exchangeable calcium or hydrogen were introduced in their complexes, in accordance with the purpose of the experiment.

The mineral exchange complex. A general increase of PO_4 adsorption is observed with the increase of the concentration of PO_4 in the solution. In the case of comparatively low concentrations the amount of adsorbed PO_4 for H-soils and Ca-soils increases gradually. Beginning with a definite concentration, the quantities of adsorbed PO_4 distribute themselves into groups. Each group includes approximately equal amounts of adsorption.

In the H-soils there are four distinct groups representing four definite forms of adsorption. The amounts of adsorbed PO_4 for these four forms are equal in milliequivalents to about 1.0, 1.5, 2.0, and 2.5 times the exchange capacity of the soil sample respectively.

For the Ca-soils five forms of adsorption are established. The amounts of adsorbed PO_4 for them are equal in milliequivalents to about 1.5, 2.0, 2.5, 3.0, and 3.5 times the exchange capacity of the soil sample, respectively. These quantities are true only for soils the complex of which is completely saturated with calcium.

The existence of the different forms of adsorption is connected with the phenomenon of adsorption of phosphoric acid ions of different valences.

The adsorption of PO_4 by the soil complex proceeds on the principle of an equivalent anion exchange. The amount of adsorption depends on the anion exchange capacity of the soil complex (which is equal to the base exchange capacity) and on the valence of the phosphoric acid ions which take part in the adsorption.

In the first form of adsorption for the H-soils, there occurs an equivalent exchange between the exchangeable hydroxyls of the complex and the trivalent PO_4 ions. In the second form of adsorption, an equivalent exchange between the exchangeable hydroxyls of the complex and the divalent HPO_4 ions is proposed. For the third and fourth forms of adsorption there occur an equivalent exchange between the exchangeable hydroxyls of the complex and, probably, a combination of phosphoric acid ions of the various valences.

The first form of adsorption for Ca-soils is typical of comparatively low concentrations of ammonium phosphate solutions. In this form the amount of PO_4 adsorption is regulated only by the amount of the exchangeable calcium in the complex. Two milliequivalents of exchangeable Ca fix three milliequivalents PO_4, i.e., the ratio of $\frac{\text{m.e. exchange Ca}}{\text{m.e. adsorbed } PO_4} = 0.666\ (0.67)$. This ratio is established for Ca-soils as well as for the soils partially unsaturated with calcium and for soils the amount of exchangeable calcium of which exceeds the exchange capacity of the soil, established at pH 7.0. In the first form of adsorption, the exchangeable calcium is simultaneously connected with the exchange complex and the adsorbed phosphoric acid ion: its one valence is in an electrostatic equilibrium with one of its two hydroxyls, and the other valence with the monovalent H_2PO_4 ion.

In the other four forms of adsorption which follow the first one, the exchangeable calcium is replaced by the ammonium ions of the solution. The adsorption for these forms is twofold: (*a*) a combination of the PO_4 of the solution with the replaced exchangeable calcium in the form of an insoluble tricalcium phosphate salt, and (*b*) adsorption of the PO_4 by the $NH_4(H)$-complex, which is formed with the loss of the exchangeable calcium. The character of adsorption by this $NH_4(H)$-complex for the last four forms is similar to that which is established for the H-soils. The valences of the adsorbed phosphoric acid ions in the last four forms for Ca-soils; namely, 2, 3, 4, and 5, correspond to the valences of the adsorbed phosphoric acid ions in the forms 1, 2, 3, and 4 of the H-soils respectively.

With comparatively high concentrations of the ammonium phosphate solutions the adsorption for the H-soils and Ca-soils becomes negative.

In all the established forms of adsorption for H-soils the amount of adsorbed PO_4 changes as the pH value of the solution changes.

The changes in pH between slight acidity and high alkalinity exert no influence on the PO_4 adsorption by Ca-soils, by soils unsaturated with calcium to

a certain degree, nor by soils the amount of exchangeable calcium of which exceeds the exchange capacity of the soil, established at pH 7.0. This statement is true when the adsorption proceeds only according to the first form for Ca-soils.

In the soils partially saturated with calcium, where the adsorption proceeds partly according to the first form for H-soils, and partly according to the first form for Ca-soils, the amount of adsorbed PO_4 is influenced by the pH.

The anion exchange reaction, in the first form of adsorption for Ca-soils, proceeds very rapidly. During the first few minutes of contact between the soil and the solution, 90 per cent of the exchangeable hydroxyls which take part in the exchange reaction are replaced by the monovalent phosphoric acid ions. In the first form of adsorption for the H-soils, the exchange reaction with the trivalent phosphoric acid ions proceeds much more slowly.

The hydrogen and calcium organic exchange complexes possess practically no properties of PO_4 adsorption. With transition from acid to neutral and alkaline pH, the adsorption becomes negative.

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A NOTE ON THE DETERMINATION OF TITANIUM IN SOILS[1]

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In a study conducted on the movement and translocation of soil constituents in the profile, the problem of a speedy and accurate method of determining titanium came up. It is known that in the sodium carbonate fusion method for the determination of soil constituents, not all of the titanium goes into solution. Some remains in the residue of the SiO_2 after the HF treatment. According to Hillebrand (1), as much as one-third of the total quantity of titanium might remain in the SiO_2 residue. Results with 35 soil samples analyzed by the authors show that the TiO_2 in the SiO_2 residue varies from one-eighth to two-thirds of the total amount.

In the routine method of analysis as given by Hillebrand for minerals the R_2O_3 precipitate on the entire sample is added to the platinum crucible in which the SiO_2 has been determined, ignited, weighed, the contents fused with $KHSO_4$, and the TiO_2 determined colorimetrically by adding H_2O_2 to the acidified solution, equal to about 5 per cent H_2SO_4.

This procedure is not applicable in soil determinations because the R_2O_3 precipitate on a 5-gm. sample—the usual size sample taken in soil analyses—cannot be conveniently determined, and it would be too bulky to handle in the crucible which contains the TiO_2 residue from the SiO_2. In the routine soil analyses an aliquot is taken for the R_2O_3 determination, but the ignition of the precipitate cannot be made in the platinum crucible because the residue in it comprises the TiO_2 of the entire 5-gm. sample. The procedure was therefore modified as follows:

The residue from the SiO_2 is fused with $KHSO_4$, and the TiO_2 is determined. An aliquot of the HCl filtrate obtained in the process of stabilizing the SiO_2 is then taken, the R_2O_3 precipitated, ignited in a platinum crucible, weighed, fused with $KHSO_4$, the TiO_2 determined, the figure for the TiO_2 multiplied by the aliquot factor, the result added to the figure on the TiO_2 in the SiO_2 residue, and the sum represents the total TiO_2 in the 5-gm. sample of soil.

If the error involved in the determination of the R_2O_3, resulting from not subtracting the TiO_2 from it, is not to be reckoned with,[2] then the procedure

[1] Journal Series paper of the New Jersey Agricultural Experiment Station, department of soil chemistry and bacteriology.

[2] The error is with the Al_2O_3 figures, since the Fe_2O_3 is determined directly on a separate aliquot.

may be simplified. The TiO_2 in the SiO_2 residue is fused with $KHSO_4$, made up to a definite volume, an aliquot corresponding to that of the R_2O_3 is taken, added to the acidified fusion mixture of the R_2O_3, and the TiO_2 determined. The figure obtained is multiplied by the aliquot factor, and the result represents the total TiO_2. In this manner only one TiO_2 determination is to be made. The error caused by the TiO_2 in the Al_2O_3 figures is, however, sometimes very serious and should not therefore be neglected. To cite a concrete example: Some of the podzols in the Lakewood soil series contain as much as 1.1 per cent of TiO_2, whereas the Al_2O_3 amounts to 2.6 per cent. It may readily be seen what the error would be if the TiO_2 were not subtracted.

If the TiO_2 is to be determined on a soil, without the necessity of getting information on the R_2O_3 constituents, the procedure resolves itself into the following: One-gram sample is taken, digested with H_2SO_4, treated with HF, the residue taken up with H_2SO_4, and the TiO_2 determined.

TABLE 1

Comparison of the H_2SO_4 and $K_2S_2O_7$ fusion methods for the determination of TiO_2

SOIL LABORATORY NUMBER	TiO_2 DETERMINED BY	
	H_2SO_4 method	$K_2S_2O_7$ fusion method
	per cent	*per cent*
1	1.236	1.087
2	0.544	0.544
3	0.399	0.394
4	0.309	0.359
5	0.689	0.679
6	0.742	0.749

Sometimes it is merely necessary to determine the TiO_2 on the HCl filtrate. In such a case it was found most convenient and simple to convert the strongly colored $FeCl_3$ solution into the practically colorless $Fe_2(SO_4)_3$, and to determine the TiO_2 on that rather than to precipitate the R_2O_3, wash, ignite, and fuse with $KHSO_4$. The method was tested out on a number of samples, as shown in table 1, and it was found to check very well with the $K_2S_2O_7$ fusion method.

Hillebrand (1) states that the error introduced by Fe_2O_3, in consequence of the yellowish color of its sulfate solution, is practically negligible, unless the Fe_2O_3 content is over 5 per cent. The soils investigated contained in every instance between 5 and 6 per cent Fe_2O_3, which would entail subtracting 0.01 per cent or less from the per cent of TiO_2. On the other hand, Merwin's (2) correction for the bleaching effect of alkali sulfates, which are invariably present when the fusion method of soil analyses is used, would give a counterbalancing correction of 0.015 per cent or less varying with a TiO_2 content of 0.5 to 1.8 per cent. If the TiO_2 exceeds 2 per cent no correction is required.

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SOIL PROFILE STUDIES: VI. DISTRIBUTION OF TITANIUM IN SOILS WITH SPECIAL REFERENCE TO PODZOLS[1]

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Studies made by the senior author (7) on the movement and distribution of the soil constituents in the profile of podzol and podzolic soils brought out the importance of the respective ions in fixing the attributes of the soil type and its degree of maturity and development. Quantitative determinations of ion distribution afford a valuable index for the interpretation of the potential fertility of the soil, methods and practices of fertilization, and, under certain conditions, even tillage operations, as in the case of accumulation of bases and other constituents in the B horizon whereby subsoiling might be advisable. Among other ions titanium has been investigated, and the results obtained are the subject of this paper.

Data on Ti in soils.—Titanium is one of the very widespread elements in the earth's crust and of course also in soils. Taking the lithosphere by itself Ti is the ninth element in the order of abundance.

Of the early reports on the Ti content of soils mention should be made of the work by Dunnington (4), who collected soil samples from many parts of the world. He analyzed 80 specimens, the Ti content of which varied from 5.42 per cent TiO_2 in a dark red clay from Virginia to 0.32 to 0.63 per cent in a number of Russian chernozems.

Vernadskii (20) discusses the sources and distribution of Ti in soils and mentions the work of Dunnington as a reproach to the Russian pedologists for neglecting the Ti ion in soil analyses.

Pellet and Freibourg (15) report a 2 per cent TiO_2 content in an Egyptian soil under sugar cane cultivation. And McGeorge (10) reports a 5 per cent TiO_2 content for some normal Hawaiian soils. In some soils the TiO_2 content is as high as 34 per cent. These Ti-soils are characterized by a small quantity of Al and large quantities of ferrous iron.

Robinson (16) presents Ti data on 26 soil samples—13 surface soils and their respective subsoils. The analyses cover primarily the soils of the eastern part of the United States—southern and northeastern states. His figures show that usually the Ti content increases in the subsoil.

Somewhat later Geilmann (5) examined 30 different German soils, in which

[1] Journal series paper of the New Jersey Agricultural Experiment Station, department of soil chemistry and bacteriology.

TABLE 1

Titanium and other constituents of some soils in the United States and Canada

(From Marbut's "Transcontinental excursion" as reported by various analysts)

NUMBER	LOCATION OF SOIL	SOIL TYPE	DEPTH	TiO_2	SiO_2	Fe_2O_3	Al_2O_3
			inches	*per cent*	*per cent*	*per cent*	*per cent*
1	Cabin Creek, Md.	Sassafras silt loam	0–2.5	0.34	87.83	1.76	3.92
2	Cabin Creek, Md.	Sassafras silt loam	4–20	0.29	90.57	1.11	4.77
3	Cabin Creek, Md.	Sassafras silt loam	20–32	0.34	80.74	2.25	10.58
4	Cabin Creek, Md.	Sassafras silt loam	33–60	0.29	82.03	2.25	8.57
5	Washington, D. C.	Leonardtown silt loam	1–4	1.10	85.48	2.10	6.23
6	Washington, D. C.	Leonardtown silt loam	4–18	1.20	82.17	3.74	8.14
7	Washington, D. C.	Leonardtown silt loam	18–28	1.18	90.40	2.42	3.60
8	Washington, D. C.	Leonardtown silt loam	36+	1.04	72.33	5.75	13.87
9	Mt. Airy, Ga.	Cecil silt loam	0–4	0.91	83.19	2.58	6.91
10	Mt. Airy, Ga.	Cecil silt loam	6–30	1.05	57.58	9.51	21.53
11	Mt. Airy, Ga.	Cecil silt loam	40–65	0.68	74.59	4.18	14.66
12	Green Hill, N. C.	Cecil clay loam	1–6	1.47	58.33	6.38	20.06
13	Green Hill, N. C.	Cecil clay loam	6–40	1.43	49.95	9.31	26.54
14	Green Hill, N. C.	Cecil clay loam	40–84	2.11	44.68	11.36	27.68
15	Green Hill, N. C.	Cecil clay loam	84+	1.23	51.39	5.56	24.72
16	Ashwood, Tenn.	Hagerstown silt loam	0–12	1.69	78.14	3.61	7.24
17	Ashwood, Tenn.	Hagerstown silt loam	12–20	1.41	78.10	3.59	9.14
18	Ashwood, Tenn.	Hagerstown silt loam	20–30	1.41	74.91	4.39	12.13
19	Ashwood, Tenn.	Hagerstown silt loam	30–42	1.52	72.16	5.82	13.18
20	Purdy, Mo.	Lebanon silt loam	0–6.5	1.08	84.24	2.74	5.79
21	Purdy, Mo.	Lebanon silt loam	6.5–11.5	0.94	83.27	2.26	7.89
22	Purdy, Mo.	Lebanon silt loam	11.5–21	1.08	80.05	2.42	10.13
23	Purdy, Mo.	Lebanon silt loam	21–26	1.08	84.55	2.84	7.25
24	Fairmont, Nebr.	Grundy silt loam	0–6	0.61	71.47	3.30	10.87
25	Fairmont, Nebr.	Grundy silt loam	6–15	0.67	71.54	3.47	11.34
26	Fairmont, Nebr.	Grundy silt loam	15–36	0.62	65.43	5.69	15.26
27	Fairmont, Nebr.	Grundy silt loam	36–44	0.58	70.08	4.35	14.02
28	Fairmont, Nebr.	Grundy silt loam	44–60	0.59	68.45	4.35	14.02
29	Lincoln, Nebr.	Grundy silt loam	0–6	1.66	73.96	2.42	9.61
30	Lincoln, Nebr.	Grundy silt loam	6–13	1.44	73.81	2.50	10.54
31	Lincoln, Nebr.	Grundy silt loam	13–15	1.66	74.51	2.74	10.72
32	Lincoln, Nebr.	Grundy silt loam	15–32	1.80	60.86	3.63	22.85
33	Olmutz, Kansas	Type not stated	0–4	0.54	73.49	2.72	10.83
34	Olmutz, Kansas	Type not stated	4–22	0.60	72.83	3.08	12.00
35	Olmutz, Kansas	Type not stated	22–32	0.55	68.71	4.22	14.66
36	Olmutz, Kansas	Type not stated	30+	0.58	67.75	3.93	13.83
37	Colby, Kansas	Colby silt loam	1–1.5	0.53	72.91	3.31	11.52
38	Colby, Kansas	Colby silt loam	4–5	0.52	70.14	4.42	12.93
39	Colby, Kansas	Colby silt loam	5–9	0.49	60.69	2.64	11.99
40	Colby, Kansas	Colby silt loam	9–40	0.52	62.64	3.28	11.68
41	Nevada	Type not stated	0–1.5	0.50	64.37	3.84	13.89
42	Nevada	Type not stated	1.5–4	0.47	61.69	3.87	13.77
43	Nevada	Type not stated	5–15	0.51	57.78	3.67	12.85
44	Nevada	Type not stated	16+	0.27	38.40	2.09	7.57
45	Indian Head, Sask.	Type not stated	0–4	0.52	60.51	5.11	12.74

TABLE 1–*Concluded*

NUMBER	LOCATION OF SOIL	SOIL TYPE	DEPTH	TiO_2	SiO_2	Fe_2O_3	Al_2O_3
			inches	*per cent*	*per cent*	*per cent*	*per cent*
46	Indian Head, Sask.	Type not stated	4–20	0.52	66.21	5.25	12.85
47	Indian Head, Sask.	Type not stated	20–30	0.51	59.51	5.21	12.79
48	Indian Head, Sask.	Type not stated	30–66	0.68	52.40	6.63	16.92
49	Marion Co. Ohio	Miami clay loam	0–7	0.84	74.61	3.25	10.86
50	Marion Co. Ohio	Miami clay loam	7–14	0.91	65.18	6.08	15.93
51	Marion Co. Ohio	Miami clay loam	14–28	0.85	62.88	6.10	17.32
52	Marion Co. Ohio	Miami clay loam	28–36	0.66	56.53	4.90	13.78
53	Westport, Ind.	Miami silt loam	0–.75	1.00	71.96	2.71	7.16
54	Westport, Ind.	Miami silt loam	.75–10	0.79	78.55	2.77	9.32
55	Westport, Ind.	Miami silt loam	10–33	0.69	72.31	4.57	12.86
56	Westport, Ind.	Miami silt loam	34–42	0.65	66.26	5.06	11.52

he found from a trace to 1 per cent of TiO_2. He also reports analyses on the TiO_2 content of a number of plants, among which potato tops had shown the highest quantity of TiO_2: 0.0186 per cent of the total dry weight, or 0.269 per cent of the total ash. In tables 1 and 2 data are compiled on the Ti content of some soils in the United States and other parts of the world. Inasmuch as the available analyses permit, a cross-section of the Ti content in the important soil zones is presented in these tables. Unfortunately very few analyses were found on the distribution of Ti in the soil profile. At best some reports give data on the Ti content of the soil at various depths which are not satisfactory for the elucidation of the movement and translocation of the soil constituents in the profile.

Besides the Ti figures those on the SiO_2, Fe_2O_3, and Al_2O_3 content are presented. They might serve as a guide in establishing the reactions involved in the distribution of Ti in the soil.

An examination of the data reveals two characteristic features: 1. The Ti content within the various soil zones differs from as low as 0.27 per cent (in the Nevada subsoil and in the immature soil from Rio de Janeiro, Brazil) to as high as 4.04 per cent (in the Central African soils) and even as high as 20.02 per cent (in the Hawaiian Ti-soils, which normally contain as much as 5 per cent). Such variations must be attributed to the Ti content of the parent material. 2. The Ti content of soils, especially the lateritic and laterites, in the tropics is generally, with a few exceptions which might be explained on the basis of intrazonality of soils, higher than in the soils of the temperate climate. This is due to the active weathering in the tropics whereby the more immobile constituents, like Fe and Al, remain behind in the process of leaching. TiO_2 with its distinct amphoteric behavior (it forms metatitanates as anhydrides of a weak acid and as a cation it forms the tetravalent salts, all of which are easily hydrolyzed) reacts similarly to the R_2O_3 constituents as shown presently. In tables 1 and 2 we see that in the soils of Cuba (the Norfolk series is more closely

TABLE 2

Titanium and other constituents of soils in various parts of the world

NUMBER	LOCATION	TYPE OF SOIL OR MATERIAL	DEPTH	TiO_2	SiO_2	Fe_2O_3	Al_2O_3
				per cent	*per cent*	*per cent*	*per cent*
1†	Croatia	Terra rossa	Surface	0.37*	36.85	12.9	28.1
2	Montenegro and Rumania	Terra rossa	Surface	0.38	50.8	9.5	16.5
3	Cuba	Matanzas clay	0–3″	1.46	46.23	8.66	23.77
4	Cuba	Matanzas clay	11–69″	1.30	43.66	12.43	29.03
5	Cuba	Matanzas clay	87–98″	1.33	43.58	11.06	29.69
6	Cuba	Matanzas clay	98–156″	1.18	43.63	10.21	31.08
7	Cuba	Matanzas clay	156–168″	0.13	6.13	1.26	3.31
8	Cuba	Nipe clay	0–26″	0.80	3.28	63.04	18.46
9	Cuba	Nipe clay	26–40″	0.26	2.25	69.56	11.13
10	Cuba	Nipe clay	40–156″	0.80	1.83	71.12	12.36
11	Cuba	Nipe clay	156–192″	0.80	1.55	68.10	14.66
12	Cuba	Parent material	192+″	0.05	41.93	7.84	2.00
13	Cuba	Truffin clay	0–4″	1.74	36.21	15.13	27.16
14	Cuba	Truffin clay	7–15″	0.81	38.67	14.70	29.92
15	Cuba	Truffin clay	26–88″	0.81	39.23	14.92	31.10
16	Cuba	Norfolk fine sandy loam	0–17″	0.42	88.55	2.65	4.66
17	Cuba	Norfolk fine sandy loam	17–30″	0.56	75.58	8.54	9.67
18	Cuba	Norfolk fine sandy loam	30–38	0.57	75.10	7.57	11.34
19	Rio de Janeiro	Immature soil	A	0.27	70.88	1.47	15.89
20	Rio de Janeiro	Immature soil	B_1	0.28	67.90	2.59	18.44
21	Rio de Janeiro	Immature soil	B_2	0.45	63.54	2.46	22.14
22	Rio de Janeiro	Immature soil	C	0.13	70.23	1.37	17.22
23	Central Africa	Type not stated	Surface	2.89	61.86	5.76	19.90
24	Central Africa	Type not stated	30″	0.56	65.38	4.38	20.38
25	Central Africa	Type not stated	Surface	1.40	39.30	4.84	12.44
26	Central Africa	Type not stated	12″	3.98	37.90	14.82	28.08
27	Central Africa	Type not stated	36″	4.04	38.20	14.60	28.74
28	Central Africa	Type not stated	72″	3.61	38.94	14.98	28.63
29	Colombo	Laterite	1 meter	1.23	33.87	19.69	26.57
30	Colombo	Laterite	2 meters	0.83	46.91	7.10	25.77
31	Colombo	Laterite	3 meters	0.88	48.73	18.88	16.07
32	Colombo	Laterite	5 meters	0.91	51.24	1.04	28.84
33	Colombo	Laterite	7 meters	0.67	63.89	2.33	18.87
34	Colombo	Gneiss	10 meters	0.88	64.42	4.21	17.25
35	Switzerland	Soil	.....	0.43	70.30	4.23	12.63
36	Switzerland	Parent material	.....	0.81	66.12	5.82	14.52
37	Switzerland	Soil	.....	0.54	66.54	4.47	13.38
38	Switzerland	Parent material	.....	1.09	61.20	0.27	16.19
39	Sweden	"Barunerde"	3–13 cm.	0.53	65.42	2.51	10.17
40	Sweden	"Barunerde"	15–32 cm.	0.59	70.00	3.31	11.97
41	Sweden	"Barunerde"	100–115 cm.	0.55	72.90	3.18	12.25
42	Egypt	Sugar cane soil	Surface	2.0 after Pellet and Fribourg (15)			
43	Hawaii	Ti-soil	Surface	18.84 } after McGeorge (10)			
44	Hawaii	Ti-soil	Subsoil	20.02 } after McGeorge (10)			
45	Germany	Clay	.....	0.885 } after Blanck and Geilmann (3)			
46	Germany	Kaolin	.....	0.284 } after Blanck and Geilmann (3)			

* In 3 of these only traces of TiO_2 were reported.

† Samples 1 and 2 were adopted from Blanck (2); samples 3–18 from Bennett and Allison (1); samples 19–22 from Harrassowitz (6); samples 23–28 from Marbut (11); 29–34 from Harrassowitz (6); 35–38 from Niggli (14); 39–41 from Stremme (19) after Lundblad.

related to the yellow earths or brown earths, which are subject to the acid hydrolysis[2] of the podzolic process of soil formation), in the Cecil series of the United States, in the soils of tropical Central Africa, Hawaii, Egypt, and others the Ti content is high. The lateritic nature of the aforementioned soils is easily discerned from the figures on the SiO_2 and R_2O_3 content. On the other hand the soils of the temperate region—nos. 24–56 in table 1, the soils of Nebraska, Kansas, Nevada, Saskatchewan (Canada), Ohio, and Indiana, and nos. 35–41 in table 2, the soils of Switzerland and Sweden—show a low TiO_2 content, as compared with the tropical soils. We need not lose sight of the fact that in the tropical soils mentioned, the parent material (nos. 7 and 12, table 2) is very low in TiO_2. The high TiO_2 content in nos. 29–32, table 1—soils from Nebraska—is undoubtedly due to the high content of this constituent in the parent material.

As we attempt to evaluate the distribution of the TiO_2 in the profile the data in table 2 are of little value, except for the Cuban soils which indicate an accumulation of TiO_2 in the surface horizon, as evidenced by the Matanzas and Truffin families, using the nomenclature of Bennett and Allison (1). The Nipe, however, seems to show no specific accumulation in the surface horizon (the low TiO_2 content at a depth of 26–40 inches between two layers of a high TiO_2 content is very strange and no explanation can be offered).

From table 1, which gives analyses on some soils in the United States, a somewhat better idea about the distribution of TiO_2 at various depths in the profile may be had. Of course, it would have been much clearer, and a better interpretation would have been possible if the depths were arranged in the order of the natural horizons. As it is, some depths might include portions of A and B and C. And it is common knowledge among pedologists that there is a quantitative and in some soils also a qualitative difference in the colloidal complex of the respective horizons. Clay, for example, accumulates in the B horizon of certain soils, and the TiO_2 content of clay is higher than of any other fraction in the soil. Steinkoenig (18) presents analyses on the various separates of a number of soils, and in most cases the TiO_2 content is highest in the fine silt and clay. Robinson and Holmes (17) report an increase of TiO_2 in finer fractions of the colloids.

It will be noted that the soils which could be designated as podzolic (the R_2O_3 and SiO_2 content indicate that), such as the Sassafras, Cecil, Leonardtown, and Miami, show tendencies to accumulate TiO_2 throughout the profile in the same manner as the lateritic soils do, mention of which has been made before. And what is more significant, the highest accumulation of TiO_2 is, as a rule, associated with a high Fe_2O_3 content. We do know of the existence of ilmenite ($FeTiO_3$), and it is not at all improbable that the new formations in the B horizon of soils undergoing the podzol process of soil formation contain such a compound. Of course there are the possibilities of forming the phos-

[2] For a detailed discussion on the acid hydrolysis in the podzol process of soil formation the reader is referred to a paper by Joffe (8).

phate of Ti—$Ti(HPO_4)_2$. The similarity in accumulation of TiO_2 in podzolic and lateritic soils may be interpreted as another link in the chain of evidence on the genetic relationship of these two types of soil formation.

Experimental:—To get a clear notion of the mode of distribution of any ion we must establish the mobility properties of the ion, i.e., we must ascertain the conditions under which the ion becomes soluble, either in true solution or as in the colloidal sol state. The method used in establishing the conditions which determine the mobility of Ti was that of Mattson (13), which, perhaps, could be designated as the method of isoelectric precipitation.

The hydroxide of Ti as an ampholyte is electropositive in acid and electronegative in alkaline solutions, as may be seen from table 3.[3]

TABLE 3

The isoelectric precipitation in the Ti-hydroxide system

A. 10 m.e. $TiCl_4$ in 1000 cc. H_2O

B. 50 m.e. NaOH in 1000 cc. H_2O

SOLUTION A	SOLUTION B	FLOCCULATION		μ/SEC. 1 VOLT/CM.	pH
		After mixing	Overnight		
cc.	*cc.*				
20	7.0	Opal.	xx	+3.03	3.6
20	7.8	Opal.	xxx	+3.03	4.0
20	8.0	Slow	xxxx	+3.03	4.1
20	8.2	Rapid	xxxx	+1.16	4.6
20	8.4	Instant	xxxx	+0.61	4.6
20	8.5	Instant	xxxx	−1.21	5.0
20	8.6	Rapid	xxxx	−2.75	6.4
20	8.8	Slow	xxx	−3.03	6.7
20	9.0	Opal.	xxx	−3.03	7.4

Isoelectric pH 4.8.

It will be noted that in the presence of Cl the Ti is positive up to pH 4.6, and is isoelectric at pH 4.8. As pointed out by Mattson (13) the ampholytes as hydroxides are probably not pure hydroxides, but they contain the anion present in solution, the nature of which influences the isoelectric pH. Beginning with pH 3.6 some hydroxide forms, as shown by the flocculation data. With an increase in pH more and more of the hydroxide is formed, i.e., less Ti is in solution. With a change in charge—as the basicity of the medium increases—the Ti electronegative complex slowly increases its electrokinetic capacity and dissociates more and more as anionic complex. It is probable that it has a composition somewhat on the order of a titanate, perhaps as

[3] The headings of tables 3 and 4 illustrate the procedure. For a more detailed outline of the method the reader is referred to the papers by Mattson (13). Suffice it to say here that the charge and migration velocity of the colloidal complex were determined in the cataphoresis cell as worked out by Mattson (12).

NaTi(OH). It is thus clear that with the increase in pH beyond the isoelectric point in either direction—strongly positive at very low pH and strongly negative at high pH readings—Ti will be in solution and therefore mobile.

If we should bar any other side reactions and assume that the Ti moves in the soil profile as if it were in the hydroxide system, then it will be mobile either as a cation at very low pH values tending to become finally ionically dispersed, or as an anionic complex at high pH values. In the podzol process of soil formation where acid hydrolysis predominates the existence of titanates as in the hydroxide system is out of the question. The pH in the podzols or podzolic soils, as a rule, is far below 6.7, the point at which the anionic complex becomes soluble. As will be pointed out presently, the Ti might become mobile even at a pH below 6.7 in the presence of other anions, but for the moment we are considering the hydroxide system under which no soluble titanates are probably below pH 6.7. On the other hand we might very well expect that in the podzolic process of soil formation there would be locally in the soil acid spots, under the influence of nitrification or sulfur oxidation, where the pH is low enough to bring the Ti in solution as an electropositive ion.

In the lateritic process of soil formation where alkaline hydrolysis prevails the Ti might become mobile as an anionic complex. But apparently the reactions which prevent the mobility of the R_2O_3 constituents in the laterite profile are also instrumental in keeping back the Ti. This might explain why in most of the laterites, for which reliable analytical data are available, the Ti seems to accumulate in the surface horizon. It is probable that if any Ti appears in solution it forms in the laterite the insoluble titanates of iron, specifically the $FeTiO_3$ (ilmenite).

As has been pointed out, the possibility of Ti moving as a positive ion in the hydroxide system is within the range of reactions in the podzol profile. We must, however, remember that in the soil we have other anions, such as SiO_3, SO_4 and PO_4, which influence the mobility of such ampholytes as the R_2O_3 and TiO_2. The effect of anions on the precipitation of Fe and Al has been noted by Joffe and McLean (9), who have shown that in the presence of SO_4 the precipitation takes place at a lower pH than in the presence of Cl or NO_3 and with PO_4 the pH is still lower. It was Mattson (13), however, who explained the mechanism of this behavior.

If we examine the data in table 4 on the isoelectric precipitation of Ti in the phosphate system we note that the isoelectric pH has been displaced, in comparison with the hydroxide system, far over on the acid side. It is as low as pH 2.7–2.8. With an increase in pH the Ti complex as a negative ion is stable up to pH 6.0 and above that it again goes into solution. It is of interest to note that at pH 2.37 and 2.92 the precipitate which formed from 40 mgm. of TiO_2 in 200 cc. of H_2O and 24.2 mgm. of P_2O_5 consisted of all the Ti and only 5.16 mgm. of P_2O_5, or the molar ratio of $\frac{TiO_2}{P_2O_5}$ at these pH values is 13.7. With an increase in pH this ratio increases; at pH 3.8 the precipitate consists of all

the Ti and 4.0 mgm. of P_2O_5. At pH 6.8 both the Ti and PO_4 ions almost remain in solution as shown by the flocculation data.

From the researches by Mattson (13) on the isoelectric precipitation of the R_2O_3 it is clear that in the silicate system the isoelectric pH is slightly higher than in the PO_4 system, i.e., the PO_4 has a higher displacing power than the SiO_3, the latter having a lower dissociation constant than PO_4. One would therefore expect that in a soil containing free PO_4 and SiO_3 ions, provided both are quantitatively in the same order, the Ti would combine with the PO_4 rather than with the SiO_3. In the laterites the PO_4 and, especially, the SiO_3 ions are in circulation, but the pH environment is hardly favorable for the Ti to combine with them, since the isoelectric pH of both of these anions in combination with Ti is far below the one prevailing in laterites. It is, however,

TABLE 4

The isoelectric precipitation in the Ti-phosphate system

A. 20 cc. $TiCl_4$ (10 m.e. TiO_2 per liter)

B. 20 cc. Na_2HPO_4 (2.42 mgm. P_2O_5)

Total volume 50 cc.

NaOH	HCl	FLOCCULATION		μ/SEC. 1 VOLT/CM.	pH
		After mixing	Overnight		
m.e.	*m.e.*				
0.000	0.197	Rapid	xxxx	.....	2.40
0.000	0.00	Rapid	xxxx	+slow	2.80
0.074	0.00	Rapid	xxxx	−1.51	3.09
0.098	0.00	Rapid	xxxx	−1.51	3.35
0.123	0.00	Rapid	xxxx	−2.02	3.71
0.137	0.00	Rapid	xxxx	−3.03	4.82
0.148	0.00	Turbid	Turbid	.....	6.28

Isoelectric pH 2.9.

possible that in the presence of Ca it might form titanite, or sphene ($CaTiSiO_5$). It is probable that some of the little SiO_2 preserved in laterites exists as sphene. In the podzols, as shown in table 5, there seems to be nothing in the P_2O_5 figures to indicate any association with the Ti ion. It is probable, however, that in the presence of free SiO_3, as is the case in the A_2 and B_1 horizon, the Ti anionic complex should replace SiO_3, acting in this case as an anion. Mineralogists long ago suspected the substitution of TiO_2 for SiO_2, although they have never been able to point out the mechanism of the reactions involved. This would also explain the tendency of Ti to accumulate usually in the A_2 or B_1 horizon in the podzols and podzolic soils.

Another point to consider in the problem of the mobility of Ti in soils is the probable effect of the humate ions in the system. It is known that all soluble Ti-salts are hydrolyzed by H_2O, but in the presence of organic compounds the reaction is impeded. This means that the humates serve as protective agents

in keeping the Ti compounds in solution. The nature and conditions of the reactions between Ti and humates have not been studied, but they may be inferred from the behavior of the humates with the R_2O_3 constituents.

The humate ion complex is negatively charged and in combination with R_2O_3 is isoelectric at low pH values. The greater the concentration of humate to a fixed quantity of R_2O_3 the lower is the isoelectric pH. With a decrease of humate and an increase of R_2O_3 the isoelectric pH increases, as shown by Mattson (13). Similar reactions probably take place in the Ti-humate systems, and the isoelectric pH of the Ti-humus complex will be lower than in the corresponding Fe and Al complexes. In podzols where the quantity of soluble humus is at times high, as indicated by the movement in the profile and its accumulation in the B horizon, the Ti-humate complex would become soluble only at very high pH values or at very low pH values, since humates have a tendency to form two isoelectric points. With the total quantity of Ti generally being low, the possible reactions between Ti and humate will usually be similar to that of a high humate content in the complex. Under such conditions, for instance, 1 millimol of $AlCl_3$ and 3 millimols of Na-humate show that at pH 4.5 flocculation is instantaneous and only above that does it remain in solution; it begins to go into solution again at very low pH values. With 4 millimols of $AlCl_3$ and 3 millimols of Na-humate the isoelectric pH is between pH 4.8 and 5.8 and above that it is in solution. Which condition prevails in the soil is, of course, problematic. Some deductions might be made, but this is beyond the scope of this paper. It only brings out that under conditions of podzolization where the pH is usually low there are possibilities for the Ti to be translocated by the aid of the humus. We must also keep in mind the effect of other soluble ions like Ca and Mg which are absorbed in the base exchange complex and thereby shift the isoelectric pH.

If we turn our attention to table 5, where data are presented on a number of podzolic and two mature podzol soils, the picture of the distribution of Ti in the profile in the light of the data and discussion on isoelectric precipitation attains a new meaning and becomes more clear.

In the Lakewood podzols the slight increase in the B horizon might be attributed to the increase of the clay fraction by mechanical movement. And, as has been pointed out, the Ti content in the clay fraction is high. On the other hand the Ti increase in A_2 must be attributed to the translocation of Ti in one way or another, in the manner previously discussed in connection with isoelectric precipitation. In the process of hydrolysis the TiO_2 probably separates as rutile, which has been frequently reported in soils. There seems to be no definite correlation between the Fe and Ti distribution in the podzol profile as there is in the laterite profile. In the podzol we have a distinctly acid condition, as indicated by the low pH values. It is possible that in the complexity of reactions associated with the stability of the isoelectric precipitation the pH value prevailing in the podzol is favorable for the slight movement of Ti compounds, as outlined in the foregoing. The high humus

content in A_1 and the soluble humus compounds in A_2 would protect the TiO_2 and only at B_1 and B_2 would the compounds hydrolyze and deposit the TiO_2.

In the podzolic soils (the Dutches, Collington, and Sassafras), we note a definite accumulation of Ti in the B horizon. As in the Lakewood podzol this increase might be partially attributed to the higher clay content. If we compare the Ti content of the parent material (C horizon) and that of the upper horizons we note a distinct accumulation in the profile. In this respect the podzolic soils approach the condition existing in the laterite.

For the problem of classifying the podzolic soils in the United States, especially those which resemble the "Braunerde" and those close to the zone of the yellow earths, which in turn might be looked upon as a transition to the

TABLE 5

Partial analyses of some soils in the podzol zone

HORIZON	DUCHES LOAM				COLLINGTON LOAM				SASSAFRAS FINE SANDY LOAM			
	pH	TiO_2	Fe_2O_3	P_2O_5	pH	TiO_2	Fe_2O_3	P_2O_5	pH	TiO_2	Fe_2O_3	P_2O_5
		per cent	*per cent*	*per cent*		*per cent*	*per cent*	*per cent*		*per cent*	*per cent*	*per cent*
A0	5.8	0.581	5.01	0.167	4.9	0.470	3.19	0.208	4.6	1.584	1.48	0.086
A1	6.8	0.679	4.78	0.204	4.7	0.556	3.67	0.168	4.8	1.787	2.37	0.031
A2	5.4	0.758	4.98	0.103	5.0	0.495	4.24	0.110	4.6	1.790	2.43	0.016
B1	5.8	0.749	6.62	0.140	4.9	0.625	5.98	0.154	4.7	1.592	2.73	0.024
B2	6.1	0.771	7.18	0.119	4.9	0.445	6.58	0.150	5.2			
C1	6.4	0.538	6.57	0.158	4.9	0.394	7.37	0.153	5.2	1.466	2.78	0.003
C2	...	.....	....	.....	4.9	0.359	6.78	0.073	...	.....	....	.....
					LAKEWOOD SAND (FULLY DEVELOPED PODZOL)				LAKEWOOD SAND (DWARFED PODZOL)			
A1	...	.....	....	.....	4.8	1.064	1.3	.....	4.2	0.544	0.72	.....
A2	...	.....	....	.....	4.8	1.039	1.1	.....	4.6	0.594	0.69	.....
B1	...	.....	....	.....	5.1	1.088	1.9	.....	4.9	.....	1.23	.....
B2	...	.....	....	.....	5.2	1.190	1.9	.....	5.0	0.544	1.66	.....
C	...	.....	....	.....	5.2	0.915	1.35	.....	5.2	0.544	1.01	.....

lateritic process of soil formation, the Ti distribution in the soil profile may serve as another link in the relationships existing between the various soil zones.

In the distribution of any soil constituent in the profile the rôle of the colloids is of prime importance. Colloid analyses complete the picture obtained from data on total analyses of the soil as a whole. Unfortunately very few systematic studies have been made on the colloids of the various soil zones by the profile method. But from the data available on the colloids of soils and subsoils in the various parts of the United States some deductions might be made. For this purpose table 6 has been compiled from analyses made in the Bureau of Soils by Robinson and Holmes (17).

It will be noted that in the colloids of the soils from Georgia, North Carolina,

Mississippi, and Kentucky the TiO_2 content in the surface soils is invariably higher than in the subsoils. The reverse is true for the soils from Maryland, New York, Indiana, Iowa, and Nebraska: the TiO_2 content is lower in the colloids of the surface soil than in the subsoil. A general deduction suggests

TABLE 6

TiO_2 in colloid fraction of various soils in the United States

SOIL TYPE AND LOCATION	DEPTH	TiO_2
	inches	*per cent*
Cecil clay loam, Ga.	0– 9	0.62
	9–18	0.52
Norfolk fine sandy loam, N. C.	0– 8	0.79
	12–36	0.71
Orangeburg fine sandy loam, Miss.	0–10	0.54
	10–36	0.44
Clarksville silt loam, Ky.	0–10	0.81
	10–36	0.63
Sassafras silt loam, Md.	0– 8	0.63
	8–22	0.70
Huntington loam, Md.	0– 8	0.40
	8–30	0.47
Chester loam, Md.	0– 8	0.58
	8–32	0.70
Ontario loam, N. Y.	0–12	0.38
	12–22	0.56
Carrington loam, Ia.	0–12	0.47
	15–36	0.65
Marshall silt loam, Nebr.	0–14	0.48
	14–36	0.50
Miami silty clay loam, Ind.	0–10	0.70
	10–30	0.79
Wabash silt loam, Nebr.	0–15	0.49
	15–36	0.56

itself: in the yellow and red earths of the south-central and southeastern states, which represent a transition from the podzol to the lateritic type of soil formation, we have an accumulation of TiO_2 in the surface soil. As we approach the Braunerde, the podzolic soils, there is a reversal in the TiO_2 content of the re-

spective surface and subsoil layers. This condition seems to prevail in the prairie soils, the degraded chernozems, of Indiana, Iowa, and Nebraska.

SUMMARY

Data are presented on the TiO_2 content of soils in the various parts of the world showing that the distribution of TiO_2 within the soil profile varies with the soil zones. It seems that in general the TiO_2 as an ampholyte behaves somewhat similarly to the R_2O_3 constituents. In the laterites the TiO_2 accumulates in the surface horizon. Similar tendencies are noted in the podzolic soils.

The mobility properties of TiO_2 have been inferred from studies on the isoelectric precipitation of TiO_2 in the hydroxide and phosphate system, following the methods inaugurated by Mattson. From the latter's work on the isoelectric precipitation in the R_2O_3 silicate and humate systems inference has been made on the behavior of TiO_2 in similar systems.

Data on the TiO_2 content in podzols and podzolic soils show that the TiO_2 is not mobile to any large extent. Comparisons of the TiO_2 content in the respective horizons in the profile bring out clearly that this constituent accumulates in the profile. Within the profile in the soils of the podzol zone there is an accumulation of TiO_2 in the B and sometime in the A_2 horizons. The probable reactions in the light of the isoelectric precipitation of TiO_2 in the lateritic and in the podzol process of soil formation are discussed.

Data are presented and discussed on the TiO_2 content in the colloid fractions of the surface soils and subsoils in several soil zones. It is brought out that in the zone of the yellow earths and red loams, the transition zone between the podzol and laterites, there is an accumulation of TiO_2 in the colloids of the surface soil, whereas in the podzolic and degraded chernozem zones there is an accumulation of TiO_2 in the colloids of the subsoil.

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